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## Nasal Eosinophilia in So-called Bacterial Hypersensitivity in Asthmatic Children

### Preliminary Report

by KJELL AAS

The question of bacterial hypersensitivity in bronchial asthma has occasioned much controversy. Some allergists claim that bacteria or parts of the bacteria act as genuine antigens. Others believe that infections act solely as provocative factors. The former school of thought advocates the use of bacterial vaccines, while the latter is more negative in approach to such treatment. This controversy is partly a result of our inadequate methods for proving or disproving the existence and/or significance of bacterial hypersensitivity.

In the Children's Department of the Oslo University Hospital some investigations related to these problems are being carried out. This is a preliminary report of some of the results.

A central question raised is whether any patients with bronchial asthma show specific allergic reactions to bacterial vaccines, i.e. do the bacterial vaccines in use contain any significant antigen?

Theoretically an increase of the eosinophilia in the nasal secretion after application of a test extract may signify that the extract carries antigenic qualities unless

it is strongly irritative to the mucosa. This seems true in specific allergies. Corresponding reactions could be expected in possible bacterial hypersensitivities causing bronchial asthma at least in the proportion of cases in which the mucosa of the nasal cavity is representative of the bronchial mucosa as regards eosinophilia.

In acute exacerbations of bronchial asthma an increase in nasal eosinophilia is commonly found. The exacerbation may be regarded as a positive reaction to unwanted and unplanned provocations having much in common with planned ones. Parallels may be drawn.

After numerous examinations for nasal eosinophilia in connection with provocation tests in specific hypersensitivities, we have started a search for corresponding reactions toward the bacterial vaccines commonly used.

### Material

For practical purposes we started to examine specially selected patients. This was necessary principally because the patients had to be closely observed in the Hospital for some time. For the examinations, those patients have been picked who presumably

suffered from "bacterial allergy" satisfying the following conditions: (1) recurrent attacks of bronchial asthma following upper respiratory tract infections such as common colds etc., (2) tendency to attacks or exacerbations of the allergic symptoms within the first few days of such infections, (3) no traceable specific hypersensitivities that may be connected with the patients symptoms (after thorough examinations including skin testing and provocation procedures).

Of 153 asthmatic children seen in the Out Patient Department or hospitalized during a 8 months period, 29 patients were selected as satisfying the criteria mentioned. Sixteen children were under 4 years of age and fourteen of these were considered unfit for the studies. These omitted patients however were examined with respect to their reactions to vaccine injections. Fifteen patients fulfilled all conditions stated and were tested with the methods described (Group A).

Parallel to these, the same methods were used in testing 13 other asthmatic children with known specific hypersensitivities (Group B).

An additional group of 16 randomly selected asthmatic children was tested with single applications. These latter patients suffered from partly known specific hypersensitivities and in most of the cases the asthmatic attacks were precipitated by common colds (Group C).

### Methods

From the older children the secretion was easily obtained by allowing the patient to blow the nose in a soft plastic "handkerchief", and partly by gently swabbing the nasal mucosa with a thin cotton-tipped metal string. When the swab was used, it was firmly pressed against the mucosa of the posterior nasal cavity for a minute or so. The latter method was uniformly employed in the youngest age groups.

The secretion was spread on a slide with as few manipulations as possible, dried in air without heating and stained with the May-

Grünwald stain for 3-5 minutes. To obtain optimum staining buffered water with a pH 5.8 was used as a solute. The slide was examined under the immersion oil lens and with optimal light conditions. For analysis the examiner must rely on a method of grading the amount of eosinophilia. A scale modified after Hansel, Andersen & Koch was found valuable (1, 7).

Nasal secretion was obtained several times during the first two days of the investigational period. From each swabbing at least two slides were made. The eosinophilia was judged, and the slide with the highest grade picked as control for the later ones.

Local application: One nostril was tamponed, gently but firmly, and the tampon was repeatedly saturated with the fluid in question. The fluids employed in this series were: *Controls* (1) 0.9 % sodium chloride, (2) Coca solution, (3) pollen- or house dust extracts, (4) "vaccine" made of indifferent diptheroids; *Test series*: (5) anticatarrh vaccine SIFF (stock vaccine), (6) staphylococcus aureus vaccine Bencard, (7) autogenous vaccine containing staphylococcus aureus or vaccine of isolated staphylococcus aureus from the patient in question. For the nasal applications all vaccines were employed undiluted, and the fluids applied in the sequence mentioned.

The tampon was removed after a few hours (usually 3-5) and was as a rule found to be covered with mucus which was used for examination. Secretion was at the same time obtained by the methods described, and this was repeated at intervals during the following 48 hours. A new tamponade was then done unless a persisting increase in the eosinophilia had been found. In the latter case no new tamponade was done until repeated checks showed an eosinophilia at the starting value or lower.

*Systemic application.* Finally the patient received a subcutaneous injection of fluid no. 4 with subsequent examinations of the nasal secretion during 4 days, and on the 5th day an injection of fluid nos. 5, 6 or 7 was given followed by nasal swabbings. The dose given in injection was variable. Those pa-



tients who showed no eosinophilic response on local applications were given 0.5 ml of the 1/1 or 1/10 dilution. Those with presumed positive reactions were given 0.5 ml of dilutions of 1/10000. To save hospitalization time the completing tests had to be performed while ambulatory in a few cases. None of these belonged to the positively reacting group.

### Results

Of the 15 patients in Group A, 3 had to be omitted because of persistent high-grade eosinophilia during the entire hospital stay. Two other children showed increased eosinophilia on the control extracts and not on the test extracts. These reactions were found after application of house dust extract and Coca solution respectively, partly giving a clue to specific hypersensitivities not formerly found in these patients. Seven of the remaining patients showed no eosinophil response whatsoever.

Three patients, however, reacted with a significant eosinophilic increase after application of the test fluids and not on the controls. The positive responses were reproducible. In two of the three patients a marked rise in the secretion eosinophilia was also found after the injection of autogenous vaccine and stock vaccine respectively. The maximum rise here was found after 36 and 48 hours. Focal reactions including sibilous sounds on auscultation were encountered in the same two patients.

In the third patient no change in eosinophilia was found after the injection and no focal asthmatic reaction was observed following it. This patient, however, acquired an otitis media of the "catarrhal"

type on the tested side 24 hours after the local application of the stock vaccine.

It is noteworthy that the patient with the most marked eosinophilia (and focal reactions) had received stock vaccine injections regularly during the preceding three years with several instances of moderate to severe asthmatic reactions following even minute doses.

In Group B, none of the 13 children reacted to the bacterial vaccines. Among the 5 patients suffering from known hypersensitivity to house dust, four showed increased nasal eosinophilia after the house dust application. One of these patients suffered from slight asthmatic symptoms including scattered sibilous sounds on auscultation the day after the bacterial vaccine injection (dose 0.5 ml of the 1/10 dilution). A slight temperature rise and some tenderness at the injection site was observed in several instances following the bacterial vaccine in this group, as well as in the other groups. A similar reaction was found after fluid number 4 as well. In no case in Group B was an eosinophilic response found after the injections.

The Group C patients were tested with intranasal vaccine applications only. Control applications were not done. Fifteen patients showed no eosinophilic response. One child, however, clearly reacted positively on the first stock vaccine application. When retested no change in eosinophilia could be found on repeated examinations. The eosinophilia first encountered may have been caused by accidental circumstances such as chance dust exposure or a slight infection.

The 14 children under 4 years given stock vaccine injections reacted with

some tenderness locally and/or a slight temperature rise. None of them reacted with exacerbations of the allergic disease.

Several of the children in all groups had formerly received stock vaccine injections, some undiluted and others in minute doses. No significant differences between these and the unvaccinated ones could be found except in the one positive child in Group A.

### Discussion

The etiologic diagnosis of "bacterial allergy" is for the present time not well based. More objective criteria should be sought for. Bergquist has experimented with combined extracts from mucus membranes and bacteria (2). The experiments are encumbered with some weak links however and ascertained results are still awaited. The leukocyte incubation method of Blatt (3) is applicable in the determination of bacterial cellular antigenicity of the delayed reaction type. In bronchial asthma the tissue response is of the atopic immediate reaction type, and no conclusions can be drawn from the cellular antigenic qualities of bacteria (4).

Our method has weak links as well. On the one hand, technical difficulties are met with. Eosinophilia in secretions is very often difficult to judge quantitatively. Counting in percentages is not satisfactory, and a rough estimate must frequently be relied on. The increase in eosinophilia noted in our study has been so convincing, however, that the examiner is left without doubt. On the other hand, some uncertain eosinophilic changes have occurred in the "negative" group. Such uncertain changes have not resulted in

any sort of hypothesis here although some of them might have some significance.

Rajke (8) claims that the nasal mucosa as an allergic responding organ is not representative for the mucosa behind the nasal cavity, including the bronchial tree. Certainly it is true that increased nasal eosinophilia does not necessarily indicate an allergic state in the bronchi. We are nevertheless convinced that in children the allergic state of the bronchi most frequently is associated with an allergic reactivity of similar quality in the nasal mucosa. This opinion is obviously shared by many allergists, and Ryssing has recently shown that clinically observable allergic manifestations in the nose and bronchi combined are found in 67 % of children on provocative testing (9). In bronchial hypersensitivities of a specific type the nasal mucosa most frequently reacts allergically to the antigen in question. A study in this Hospital of the frequency of nasal eosinophilia in asthmatic children is in progress. One hundred and eighteen patients are hitherto included in this series. Of the children suffering from severe asthma every one shows nasal eosinophilia. Seventy-eight % of those with moderate asthma and about 60 % of those with mild asthma show eosinophilia in the nasal secretion. Forty-eight patients were examined in this respect when having symptoms and all but four showed pathological nasal eosinophilia.

More questionable is whether the positive reactions described in connection with the vaccine applications indicate that the patient suffers from a true bacterial hypersensitivity. In the nasal cavity bacteria are normally found in astronomic numbers and if one accepts the hypothesis of signi-

ficant bacterial antigenicity, these bacteria theoretically may be responsible for the pathological secretion with eosinophilia found even between asthmatic attacks. We find difficulties in explaining how the application of proportionately inconsiderable amounts of bacteria may release an allergic reaction. The fact that the reaction is reproducible with the bacterial vaccine while not with the controls suggests nevertheless that the vaccine employed contains an antigen of significance. Changes forced on the bacteria through the vaccine preparation process as for example crushing, decomposition or release of incorporated antigenic substances may theoretically play a role. Non-specific protein components must also be taken into consideration.

A specific allergic reaction toward the bacterial vaccines could easily be explained in patients who previously had received similar vaccine injections. In such cases an assertion that the vaccinations contrary to the purpose had sensitized the patient, could rest uncontradicted. Examples of such appropriate sensitizing effects are known to most allergists (5). One of our patients lends himself to such argumentation, but the other two do not.

Non-specific irritations (thermal, mechanical and chemical) may cause an increase in the secretion eosinophilia as well as clinical exacerbations of the al-

lergic disease. The positive reactions described may be due to irritative properties of the test solution. When such reactions are not observed in the control experiments, as for example after the application of diptheroid vaccine, it is tempting to assume that the "irritation" is synonymous with "antigenic quality".

This assumption is supported by the findings of eosinophilic increases after the findings of eosinophilic increases after the parenteral injections of the vaccine that showed activity on nasal applications, compared to the failure to respond to control fluid injections. Support is also found in the observation of asthmatic symptoms provoked by test vaccine injections in minute doses.

The observations obtained stimulate further investigations in this important field.

### Summary

A preliminary report is given of some studies concerning bacterial vaccines in asthmatic children. A method is introduced in which nasal eosinophilia is studied in connection with local applications of bacterial vaccines and controls. A reproducible positive response is found in three patients of a selected group suffering from presumed "bacterial allergy". In two of these patients focal asthmatic reactions are observed following the injection of minute doses of the vaccine in question. The method is briefly discussed.

### References

1. AAS, K.: Eosinofili i sekret. *T Norske Lægeforen*, 21: 1237, 1959.
2. BERGQUIST, G.: A contribution to the problem of autosensitization in bronchial asthma. *Acta Allerg*, 10: 187, 1956.
3. BLATT, H.: The identification of specific allergens in bacterial allergy and their use in desensitization. *Ann Allergy*, 10: 53, 1952.
4. FEINGOLD, B. F.: Infection in bronchial allergic disease. *Pediat Clin N Amer*, 6: 709, 1959.

5. FLENSBOG, S. W.: Om formentlig bakteriell-allergisk asthma hos børn og dennes behandling. *Ugeskr Læg*, 112, 12: 387, 1950.
6. GLASER, J.: Allergy in Childhood, C. C. Thomas. Springfield 1956.
7. KOCH, H.: Allergical investigations of chronic otitis. *Acta Oto Laryng (Stockh) Suppl*, 62: 1947.
8. RAJKE, E.: Allergie und allergische Erkrankungen. Budapest 1959.
9. RYSSING, E.: The results of provocative tests with inhalant allergens in asthmatic children. *Acta Allerg*, 14: 433, 1959.

Received March 7, 1960

Accepted April 28, 1960

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## Precipitating Antibodies in Human Sera from Different Age Groups and in Colostrum as Determined by Streptococcal Antigens with Diffusion-in-Gel Methods

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The development of the serum proteins which show antibody activity, also called the immune globulins (13, 14), have recently been studied by immune electrophoresis (24, 25). It has been observed that  $\gamma$ -globulins appear in the serum of the fetus after 12 weeks of pregnancy. The  $\beta_{2A}$ - and  $\beta_{2M}$ -globulins which also belong to the immune globulins are not found in the serum of the fetus and newborn but they appear during the first weeks after parturition. The  $\gamma$ -globulins of the newborn are derived from the mother via the placenta as shown by studies with labelled  $\gamma$ -globulins (19, 20). Due to the disappearance of these  $\gamma$ -globulins the level in the serum of the child decreases after birth. At about the age of three months an increase of the  $\gamma$ -globulin level can be observed as the  $\gamma$ -globulin of the child counteracts the elimination (27). The  $\gamma$ -globulin level then slowly increases to the adult value.

This investigation is an attempt to reflect this development of the immune globulins by a study of precipitating antibodies against  $\beta$ -hemolytic streptococci in different age groups—fetal, newborn, child and adult.

### Material

*Antigens.* Filtrates of 18 hour cultures of  $\beta$ -hemolytic streptococci (strain S 84, Lancefield group A, Griffith Type 3) grown in beef heart infusion broth have been used. These antigen preparations were concentrated about ten times by lyophilization or by pervaporation before use.

*Antisera.* An immune serum from a horse immunized with  $\beta$ -hemolytic streptococci (strain Dochez N Y-5, Lancefield group A, Griffith Type 1) was used as a reference serum. Immune sera from sheep immunized with human blood plasma were used to localize the different protein fractions of the electro-separated materials in the immune electrophoretic analyses.

*Patient material.* Umbilical cord sera were obtained from legal and spontaneous abortions after 13–40 weeks of pregnancy as well as at full term deliveries. Sera were taken from the corresponding mothers. The material also included sera from children varying in age from a few days to 16 years. Sera from normal adults were used as a comparative material.

All of the sera tested were taken from individuals without any recent history of streptococcal infections. The antistreptolysin O titer was determined and sera with a titer of 200 Todd units/ml or less were chosen. In some sera, mainly the fetal sera, the titer was not determined.

Sera from individuals with an antistrepto-

lysins O titer of 400 Todd units/ml or more were analysed for comparative purposes.

Colostrum samples were taken from some of the mothers within one day of parturition.

### Methods

The *double diffusion-in-gel* analyses were performed according to Ouchterlony (28), as slightly modified by Hanson (9). The micro modification developed by Wadsworth (32) was also used to some extent. The *immune electrophoretic technique* of Grabar & Williams (5, 6) was employed in the modification reported by Wadsworth & Hanson (33). To demonstrate antibodies present in too low quantity to give visible precipitates with the standard immune electrophoretic technique a specially devised *comparative immune electrophoretic technique* described by Wadsworth & Hanson (33) was used. The antistreptolysin O determinations were performed at the Bacteriological Laboratory, Sahlgren Hospital. The streptolysin O preparation used for the determinations was the same as the reference antigen employed in this study. In the routine method of this laboratory the results are recorded as titers 50, 100, 200 etc. Todd units/ml.

### Results

In the comparative double diffusion experiments a reference spectrum was used which was formed by the streptococcal antigen and the reference horse immune serum. The spectrum consisted of twelve lines which were numbered I–XII (Fig. 1). Eleven of these were demonstrated to be separate serological systems, while systems IX and X seemed to be serologically related. Some of the antigenic factors represented by these lines have been identified with known streptococcal antigen preparations (16). Thus the lines I–IV were found to be con-

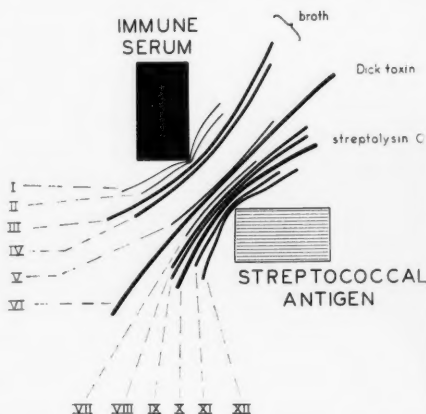


Fig. 1. Diagrammatic representation of the reference spectrum formed by the streptococcal antigen preparation and the reference serum. The precipitation lines are numbered I–XII and the identified systems are indicated.

nected with antigenic factors in the broth used for the cultures. The line VI identified with a purified Dick toxin, the line X with a pure streptolysin O preparation and the line XI with streptococcal DNase. The reference spectrum did not show any line correlated to the M-antigen. The comparative double diffusion analyses of the sera from fetuses, newborn and mothers with the reference spectrum were performed in five basin plates. As may be seen in Fig. 2 the antigen was placed in the two lower basins, the reference serum in the central upper basin and the two sera from the fetus or newborn and the corresponding mother were placed in the basins on each side of the reference serum basin. The sera from children and adults were compared with the reference spectrum in three basin plates.

Several of the precipitating antibodies in the analyzed sera could be identified by comparison with the reference spec-

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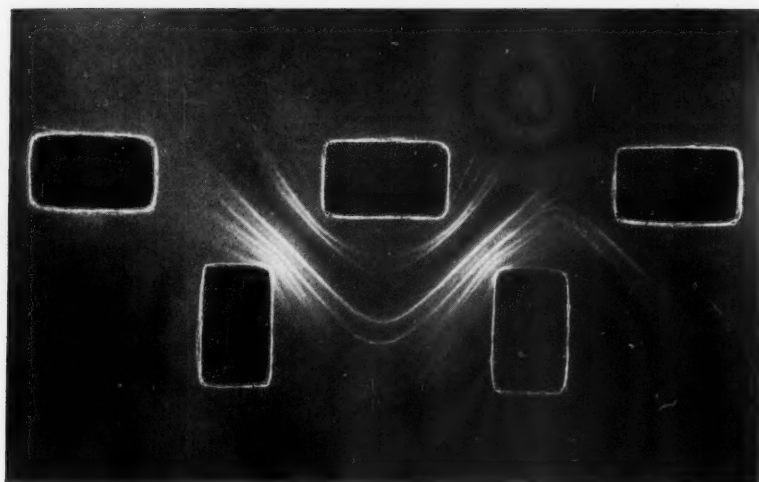


Fig. 2. Photo of a comparative double diffusion analysis where the sera from a mother (right upper basin) and her child (left upper basin) are compared with the reference serum (central basin) by means of the streptococcal antigen (lower basins).

TABLE 1. Serum components demonstrated in blood sera from different age groups by comparative analysis with the reference spectrum

Age groups	Identified serum components												Unidentified components	Number of cases
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII		
13-17 weeks														9
18-20 "								4	3	6	4	3	4	16
21-40 "					2			7	7	8	5	5	8	9
Newborns					1	1	1	12	11	14	8	6	11	15
0-2 months					2			3	6	5	2	2	10	13
3-8 "								1	2	4		1		15
9-17 "														13
1.5-2 years								1	2	1	1		2	18
3-5 "					1			3	5	6	5	1	4	21
6-8 "							1	5	5	7	2	3	8	11
9-12 "					1	1	1	9	11	14	8	10	7	18
13-16 "					1	1	1	9	14	16	12	11	11	19
Adult					9	1	1	26	23	32	13	18	19	32
Total					17	4	6	80	89	113	60	59	83	209

trum (Table 1). The lines I-IV which corresponded to antigenic factors in the broth did not identify with any lines formed with the human sera. Antibodies corresponding to the systems V, VI and VII

were only found in a few cases, while antibodies corresponding to the systems VIII, XI and XII were found in about a third of the examined sera. The precipitation lines IX and X, which may represent only one

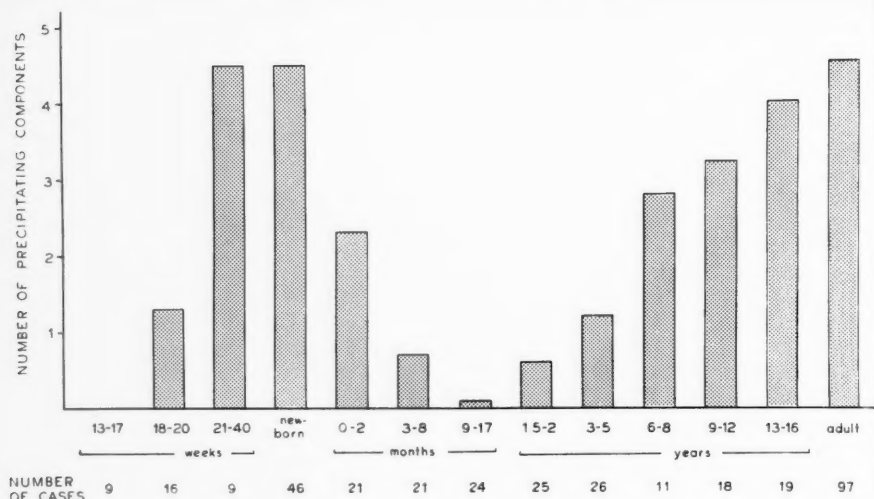


Fig. 3. Graphic representation showing the average number of precipitating antibody components found in 342 human blood sera from different age groups. (This material includes 133 sera which were compared with another antiserum and therefore could not be inserted in Table I.)

serological system, were the most frequently identified systems in the analyzed material. Some of the demonstrated precipitation lines could not with certainty be identified by means of the used reference serum. Qualitative differences between the identified antibody components in the various age groups could be noted. Differences between the total number of precipitating antibodies demonstrated in these groups were also observed (Fig. 3). In the fetal material taken after 13-17 weeks of pregnancy no serum contained precipitating antibodies against  $\beta$ -hemolytic streptococci, but after 18-20 weeks of gestation the fetal sera averaged 1-2 precipitating antibodies. In the group of fetuses aborted after 21-40 weeks of pregnancy an average number of 4-5 precipitating antibody components was found. The same number was found

in the umbilical cord sera from newborn delivered at full term. A decreasing frequency of antibodies was found after birth, and the lowest number was obtained in sera from children 9-17 months of age. After the age of 1.5 years the average number of antibodies gradually increased to the same figure as found in the adult material.

The comparison between the antibodies in the sera from the mothers and the corresponding umbilical cord sera from the fetuses and newborn gave some information. The sera from the mothers of the fetuses aborted after 18-20 weeks all contained a large number of antibodies while in only half of the corresponding fetal sera precipitating antibodies were demonstrated. The average number of antibody components in the fetal sera from this age group was lower than that found in the

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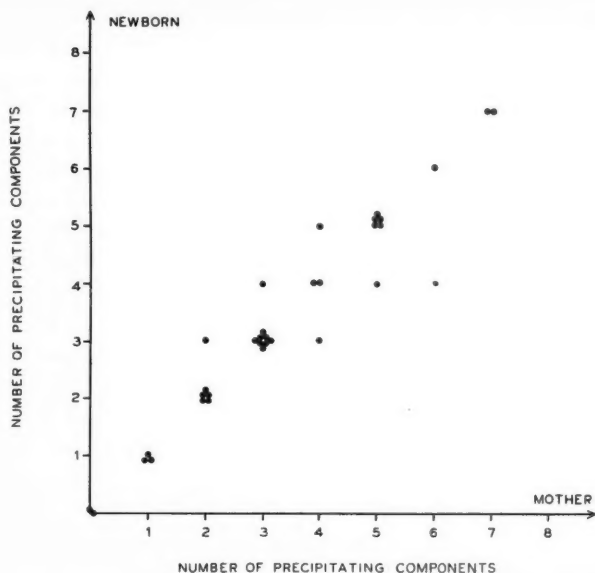


Fig. 4. Diagram showing the correlation between the number of precipitating antibody components found in the blood sera of 34 mothers and their newborn children.

mothers' sera (Fig. 3). The comparison in the next age group of 21-40 weeks of pregnancy indicated that the same number of antibodies was found in the corresponding maternal and fetal sera. This correlation was also demonstrated by the fact that the comparative analyses with the reference spectrum identified mainly the same antibodies in the maternal and fetal sera. A similar comparison between the sera from newborn and their mothers showed a perfect positive correlation regarding the number as well as the identity of the demonstrated lines (Fig. 4). In 15 of these cases antistreptolysin O determinations were performed. No difference could be found between the mothers and their offspring.

The relation between the titer of the single antibody, antistreptolysin O, and

the more complete antibody content as determined by the Ouchterlony technique was studied. An increasing number of precipitating antibodies was found with an increasing antistreptolysin O titer as seen in Fig. 5.

A possible source of immune globulins for the child may be the mother's milk. For this reason colostrum samples were analyzed for the presence of antibodies revealed by our streptococcal antigen with the diffusion-in-gel techniques. In the 42 samples analyzed precipitating antibodies were demonstrated in about 50 %. Only one antibody component was found in most of the colostrum specimens and the same antibody among several others was seen in the blood serum of the mother.

Immune electrophoretic studies were

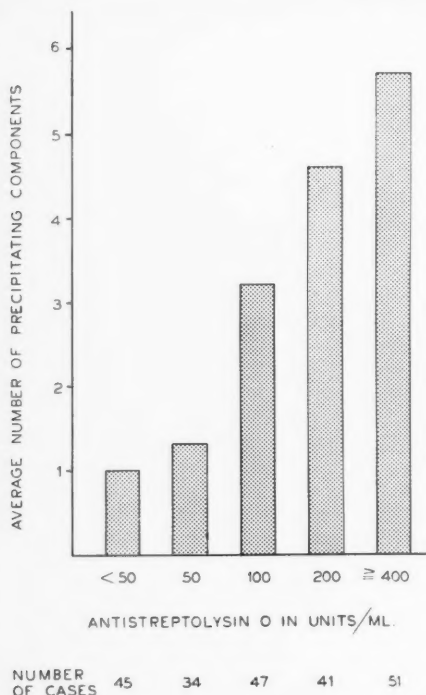


Fig. 5. Graphic representation showing antistreptolysin O titers correlated to number of precipitating antibody components in 218 human sera.

included in the investigation in order to determine the electrophoretic characteristics of the demonstrated immune globulins. Some sera from each age group were examined and altogether 45 sera were analyzed in this respect. The sera were electrophoretically separated and thereafter analyzed with the streptococcal antigen (Fig. 6). The position of the different serum protein fractions after the electrophoresis was determined by an anti-human plasma immune serum. In some instances the comparative immune electrophoretic technique had to be used in order to demonstrate the precipitating serum antibodies. They were found to be localized mainly in the  $\beta_2$ - and in some cases also further out into the  $\gamma$ -globulin region. In a few cases the precipitates were localized more toward the  $\beta_1$ -globulin area. No consistent differences could be observed between the precipitates obtained with the sera from the various age groups. Some colostrum samples were also analyzed in this way. As a rule it was not possible to demonstrate the antibodies in

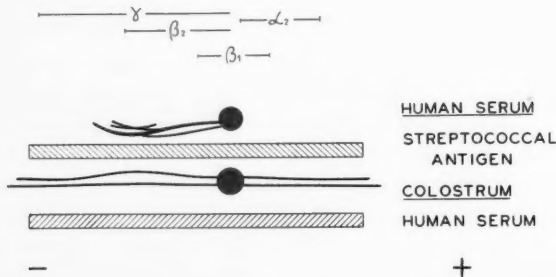


Fig. 6. Schematic representation of an immune electrophoretic analysis of human serum and a comparative immune electrophoretic analysis of colostrum showing the localization of streptococcal antibodies. The electro-separated serum and colostrum were analyzed with a streptococcal antigen. The colostrum was supplemented with a serum containing streptococcal antibodies in order to obtain a visible precipitate. The localization of the serum fractions, as indicated in the upper part of the figure, was determined with an anti-human plasma immune serum. (The separated materials are underlined.)

the colostrum with the standard immune electrophoretic technique since they were present in too low quantity to form visible precipitates. Thus the comparative modification of the immune electrophoresis was utilized. Such an analysis is shown in Fig. 6 where electro-separated colostrum was analyzed with the streptococcal antigen diffusing from a longitudinal basin parallel to the direction of the electro-separation. Additional antigenic material containing the same antibody as the colostrum in a higher concentration and diffusing from the other longitudinal basin was required to demonstrate the presence of the antibodies in colostrum. The antibodies against  $\beta$ -hemolytic streptococci found in colostrum were mainly localized in the  $\beta_2$ -globulin region.

### Discussion

The problems concerning the transfer of antibodies from mother to offspring have been reviewed by Vahlquist (29, 30). It is there stated that antibodies are principally transferred to the fetus in utero. Vahlquist, Lagercrantz & Nordbring (31) have shown that antistreptolysin O antibodies appeared in the blood of the fetus after the middle of gestation. We found precipitating streptococcal antibodies in fetal sera before 20 weeks of pregnancy as reported in a preliminary note (15). This is in accordance with the findings of  $\gamma$ -globulins in fetal sera after 12 weeks of pregnancy (24, 25). The passage of  $\gamma$ -globulins through the placenta from the mother to the fetus is well illustrated by the good correlation we found between the antibodies demonstrated in the sera from mother and fetus

as early as after 21 weeks of gestation. This relationship persisted until at birth a perfect positive correlation was obtained when identical antibodies were found in the sera from the mother and newborn. In addition the characteristics observed in immune electrophoretic analyses were mainly the same in all of the age groups. The antistreptolysin O titers were the same in the analyzed pairs. The lastmentioned finding is not in agreement with a report by Stück & Silomon (26) and others concerning streptolysin O titers in sera from newborn and mothers but seems to be supported by the studies of Adamson, Löfgren & Malmnäs (1). The titre steps in our antistreptolysin O determination may have been too great to point up minor differences in levels of antibodies. By the diffusion-in-gel techniques it was possible to compare not only a single but several streptococcal antibodies—still no differences were noted. This may be in accord with the fact that the  $\gamma$ -globulin levels of the mother and newborn are the same (21). The only immune globulins that exist in the fetal serum before birth are  $\gamma$ -globulins, while the  $\beta_{2A}$ - and  $\beta_{2M}$ -globulins not appear until after parturition (13, 25). This means that from an immunological point of view the streptococcal antibodies are likely to be  $\gamma$ -globulins and that they are not related to  $\beta_{2A}$ - or  $\beta_{2M}$ -globulins.

After birth the elimination of the passively acquired antibodies was reflected by a decreasing number of precipitating antibodies in the sera from children. The decreasing part of the curve (Fig. 3) is presumably related to the half-life of  $\gamma$ -globulins reported to be about 20 days (3, 34). After an average value of compo-

nents which was almost zero in the age group of 9-17 months an increase was observed which illustrates the phase of the active immunization. The level of the adult was then gradually reached. Between the age groups 3-5 and 6-8 years a marked increase was noted (Fig. 3). This may reflect repeated streptococcal infections at the beginning of the school age.

Antistreptolysin O antibodies in human colostrum have been studied (18, 22). In addition by employing diffusion-in-gel methods other streptococcal antibodies have here been investigated. They were found to be localized in about the same region as the serum antibodies after immune electrophoresis. The immune globulin fraction of human milk has been shown to contain  $\gamma$ -,  $\beta_{2M}$ - and  $\beta_{2A}$ -globulins (7, 10, 11). The streptococcal antibodies in the milk belonged to the  $\gamma$ -globulins as did the serum antibodies. The antibodies in human milk are considered, however, to be of negligible importance to the child (20, 21, 22, 23, 30) although some kinds of antibodies occur in high amount in the milk and do not seem to pass the placenta to any great extent (2, 18).

A few investigators have used diffusion-in-gel methods to analyze streptococcal antibodies in human sera (4, 8, 9, 12). With sera from normal adults Halbert, Swick & Sonn found between 0 and 4 lines, Hanson found between 1 and 7 with an average of 3 to 4 lines. Bonilla-Soto & Pomales-Lebrón reported an average of 2.45 lines for a similar material. In a material of sera from individuals with streptococcal infections or rheumatic fever Halbert *et al.* found between 2 and 7 lines, while Hanson found between 2 and 9. Similar results were obtained by Harris,

Harris & Ogburn who used the Oudin technique. The present investigation on a larger material confirms the earlier results. The results of the analyses of the sera from children and newborn babies are mainly in agreement with the findings of Bonilla-Soto *et al.* obtained from a similar material. The fact that in this study a higher number of lines were found in the analyses of human sera than in the other reports may be due to differences in the used techniques and antigen preparations. Refilling of the basins in the double diffusion analyses has been done. This procedure has been reported to give rise to artefacts in some instances (17). That no such artefacts have influenced the presented results has been controlled by the use of the known reference spectrum included in all performed experiments.

Streptococcal infections during the phase of active immunization give an immune response that is reflected by the demonstrated gel precipitation patterns. The immune response seems to be the result of repeated infections over a long period of time and is therefore not remarkably affected by acute infections as shown by the diffusion-in-gel methods (9). This was also indicated by the fact that a lack of streptococcal antibodies was detected only in the sera from the youngest children while sera from adults with few exceptions contained several antibodies. That some changes in the gel precipitation patterns occurred after streptococcal infections was evident, however, as some correlation between the number of lines and various antistreptolysin O titers existed (Fig. 5). The antistreptolysin O antibody was the most frequently identified antibody in the analyzed sera. This suggests



that the antistreptolysin O determination may be a good indicator of the presence of streptococcal infections.

### Summary

The development of the immune globulins was studied by means of the determination of precipitating antibodies against  $\beta$ -hemolytic streptococci in sera from different age groups—fetal, newborn, child and adult. The analyses were performed with diffusion-in-gel methods.

In fetal sera after 18–20 weeks of gestation precipitating antibodies were found, but in a lower number than in the sera from the corresponding mothers. The same number of antibodies were found in the maternal and umbilical cord sera after 21–40 weeks of gestation as well as at parturition. The antistreptolysin O titer was also the same in the sera from the mothers and newborn. From birth till the age of 9–17 months a decreasing number of antibodies was seen. From this age a slow increase was noted and the level which was found in the adult material was ap-

proached in the age group of 13–16 years. The same immune electrophoretic characteristics was demonstrated for the antibodies in the different groups of sera as in colostrum.

Finally a positive correlation was found between the number of precipitating antibodies and the antistreptolysin O titers.

### Acknowledgements

The authors wish to thank Professors Ö. Ouchterlony and R. Zetterström for their current interest and valuable advice. We are much indebted to Assoc. Prof. S. P. Halbert, Columbia University, New York, who kindly supplied the purified preparations of streptolysin O and streptococcal DNase as well as other most useful preparations. For the umbilical cord sera we thank Dr. S. Spetz and other members of the staff at the Clinic of Obstetrics and Gynecology I, University of Gothenburg. We must also express our appreciation of the technical assistance of Mrs. A.-M. Hirschfeld, the Misses M. Wikström and B. Willnäs and thank Mrs. C. Wadsworth who kindly revised the English text. The investigation was supported by a grant from Konung Gustav V:s 80-årsfond.

### References

1. ADAMSON, C. A., LÖFGREN, S. and MALMÄS, C.: Antibodies in mothers and newborn infants. *Scand J Clin Lab Invest*, 3: 52–57, 1951.
2. ARNON, H., SALZBERGER, M. and OLITZKI, A. L.: The appearance of antibacterial and antitoxic antibodies in maternal sera, umbilical-cord blood and milk. Observations on the specificity of antibacterial antibodies in human sera. *Pediatrics*, 23: 86–91, 1959.
3. BARR, M., GLENNY, A. T. and RANDALL, K. J.: Concentration of diphtheria antitoxin in cord blood and rate of loss in babies. *Lancet*, 2: 324–326, 1949.
4. BONILLA-SOTO, O. and POMALES-LEBRÓN, A.: Variation with age of antistreptococcal antibodies and their correlation with antistreptolysin O titers in human sera. *Proc Soc Exp Biol Med*, 102: 337–340, 1959.
5. GRABAR, P. et WILLIAMS, C. A.: Méthode permettant l'étude conjuguée des propriétés électrophorétiques et immuno-chimiques d'un mélange de protéines. Application au sérum sanguin. *Biochim Biophys Acta* 10: 193–194, 1953.
6. — Méthode immuno-électrophorétique d'analyse de mélange de substances antigeniques. *Biochim Biophys Acta*, 17: 67–74, 1955.
7. GUGLER, E. und VON MURALT, G.: Über immunoelektrophoretische Untersuchungen an Frauenmilchproteinen. 2. Mitteilung. *Schweiz Med Wochr*, 89: 925–929, 1959.

8. HALBERT, S. P., SWICK, L. and SONN, C.: The use of precipitin analysis in agar for the study of human streptococcal infections. II. OUCHTERLONY and OAKLEY techniques. *J Exp Med*, 101: 557-576, 1955.
9. HANSON, L. Å.: Immunological analysis of streptococcal antigens and human sera by means of diffusion-in-gel methods. *Int Arch Allergy*, 14: 279-291, 1959.
10. — Immunological analysis of human milk. *Int Arch Allergy*, 15: 245-256, 1959.
11. — The serological relationship between human milk and blood plasma. *Int Arch Allergy*, 17: 45-69, 1960.
12. HARRIS, T. N., HARRIS, S. and OGBURN, C. A.: Gel-precipitation of streptococcal culture supernates with sera of patients with rheumatic fever and streptococcal infections. *Proc Soc Exp Biol Med*, 90: 39-45, 1955.
13. HITZIG, W. H.: Die physiologische Entwicklung der "Immunglobuline" (Gamma und Beta<sub>2</sub>-Globuline) *Helv Paediat Acta*, 12: 596-605, 1957.
14. HITZIG, W. H., SCHEIDEGGER, J. J., BÜTLER, R., GUGLER, E. and HÄSSIG, A.: Zur quantitativen Bestimmung der Immunglobuline. *Helv Med Acta*, 26: 142-151, 1959.
15. HOLM, S. and HANSON, L. Å.: The presence of precipitating antibodies against  $\beta$ -hemolytic streptococci in sera from different age groups—fetal, newborn and child. 12th Scand. Congr. of Pathology and Microbiology, Gothenburg, June 1959. *Acta Path Microbiol Scand* (in press).
16. — To be published.
17. KAMINSKI, M.: Quelques observations sur la technique de précipitation spécifique en milieu gélifié d'Ouchterlony. I. Influence de la recharge en antigène. *Bull Soc Chim Biol (Par)*, 36: 279-288, 1954.
18. MALMNAS, C., LÖFGREN, S. and ADAMSON, C. A.: Om övergång av antikroppar från modern till fostret och det nyfödda barnet. *Svensk Läkartidn*, 48: 1737-1741, 1951.
19. MARTIN DU PAN, R., SCHEIDEGGER, J. J., WENGER, P., KOECHLI, B., ROUX, J. et RABINOWITZ, J.: Le passage trans-placentaire de la gamma-globuline chez la femme. *Et Neo-natal*, 7: 71-80, 1958.
20. MARTIN DU PAN, R., SCHEIDEGGER, J. J., WENGER, P., KOECHLI, B. and ROUX, J.: Das Verhalten der intramuskulär, intravenös und per os verabreichten Gamma-globuline. *Blut*, 5: 104-114, 1959.
21. VON MURALT, G. and GUGLER, E.: Die Reifung der Immunglobuline. *Helv Med Acta*, 26: 410-423, 1959.
22. NORDBRING, F.: The appearance of anti-streptolysin and antistaphylococcal in human colostrum. *Acta Paediat (Upps)*, 46: 481-496, 1957.
23. — The failure of newborn premature infants to absorb antibodies from heterologous colostrum. *Acta Paediat (Upps)*, 46: 569-578, 1957.
24. SCHEIDEGGER, J. J., MARTIN, E. et RIOTTON, G.: L'apparition des diverses composantes antigéniques du sérum au cours du développement foetal. *Schweiz Med Wschr*, 86: 224-226, 1956.
25. SCHEIDEGGER, J. J. et MARTIN DU PAN, R.: Étude immuno-électrophoretique des protéines sériques du nouveau-né et du nourisson. *Et Neo-natal*, 6: 135-144, 1957.
26. STÜCK, B. and SILOMON, R.: Vergleichende Untersuchungen zum Antistreptolysingehalt im Serum von Müttern in der Schwangerschaft und bei der Geburt sowie von ihren Neugeborenen. *Machr Kinderheilk*, 108: 8-12, 1960.
27. ORLANDINI, O., SASS-KORTSAK, A. and EBBES, J. H.: Serum gamma globulin levels in normal infants. *Pediatrics*, 16: 575-584, 1955.
28. OUCHTERLONY, Ö.: Diffusion-in-gel methods for immunological analysis. *Progr Allergy*, Vol. 5, pp 1-78 (KARGER, Basel/New York 1958).
29. VAHLQUIST, B.: Placental transfer of antibodies in human beings. *Et Neo-natal*, 1: 31-45, 1952.
30. — The transfer of antibodies from mother to offspring. *Advance Pediat Vol. 10*, pp 305-338 (Year Book Publishers, Chicago 1958).
31. VAHLQUIST, B., LAGERCRANTZ, R. and NORDBRING, F.: Maternal and foetal titres of antistreptolysin and antistaphylococcal at different stages of gestation. *Lancet*, 2: 851-853, 1950.
32. WADSWORTH, C.: A slide microtechnique for the analysis of immune precipitates in gel. *Int Arch Allergy*, 10: 355-360, 1957.
33. WADSWORTH, C. and HANSON, L. Å.: Comparative analysis of immune electrophoretic precipitates employing a modified immune electrophoretic technique. *Int Arch Allergy* (in press).
34. WIENER, A. S.: The half-life of passive acquired antibody globulin molecules in infants. *J Exp Med*, 94: 213-221, 1951.

Received April 1, 1960

Accepted April 9, 1960

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From the Copenhagen County Hospital, Gentofte, Denmark. Department of Radiology (Directors: E. de Fine Licht and Olaf Petersen, M.D.), Paediatric Department (Director: P. W. Bræstrup, M.D.), and Department of Thoracic Surgery (Director: Ib Andersen, M.D.)

## Congenital Obstructive Emphysema in Infants

by M. EIKEN

Localized pulmonary emphysema is relatively common in infants with respiratory infections, following aspiration of foreign bodies and less common as a result of compression of a bronchus by enlarged lymph nodes or a lung cyst. However, a few cases of localized emphysema which appear to be of congenital origin have been reported.

The aetiology is generally believed to be some form of obstruction of a bronchus, the passage of air being relatively unhindered during inspiration, but inhibited or completely blocked during expiration. Thus, aspiration of viscous mucus in the course of pneumonia may cause atelectasis or emphysema, according to whether the obstruction is complete or incomplete. In congenital emphysema various forms of obstruction have been demonstrated. The commonest finding is a defective development of the cartilaginous rings in areas of the bronchial wall (4, 7, 9, 15, 16, 19, 20). In other cases the cause was thought to be a redundant mucosal flap acting as a check-valve (8, 13, 18), compression due to a patent ductus arteriosus (8), or a dilated vein (18) or stenosis without demonstrable changes of the cartilaginous tissue (10). In 24 out of the 50 recorded

cases of congenital emphysema, however, no obstruction was found (1, 3, 6, 8, 11, 12, 15, 18, 20, 22, 23, 24, 25) despite the fact that operation or autopsy had been performed in all but 7 of these cases. In 3 instances kinking of a bronchus was believed to be an aetiological factor (12, 14). That an abnormal development of the elastic tissue of the lung might be responsible in some of these cases has also been considered (23), but this presumption has never been confirmed.

A few patients have shown signs of heart disease (10, 20), two had a large defect in the anterior mediastinum (9, 14), and in one case a diaphragmatic hernia was revealed (24). As a rule, however, this form of congenital emphysema does not appear to be accompanied by other developmental anomalies.

Recently, a few cases of a special form of emphysema have been reported, i.e. obstructive emphysema comprising the entire right lung or in exceptional cases both lungs. This kind of emphysema is due to compression of the right main bronchus or of the distal end of the trachea by an anomalous course of the left pulmonary artery (5, 17, and others). This anomaly differs from those mentioned

above in being frequently associated with other vascular malformations, such as an abnormal origin of the arteries from the aortic arch, dextroposition or coarctation of the aorta, persisting left superior vena cava, or septal defect.

The clinical signs of congenital localized emphysema generally appear within the first months of life, characterized by respiratory difficulty, often with prolonged expiration, usually a dry cough, and not infrequently cyanosis. Often nutritional disturbances are the result of the dyspnoea. These signs are due to the emphysema causing a more or less pronounced compression atelectasis of the other parts of the lungs, primarily homolaterally, but often also contralaterally, the heart and mediastinum being displaced towards the good side. Infection of the respiratory tract is generally not present initially, and if so, one may doubt the congenital origin. On the other hand, secondary infection aggravating the condition is not uncommon. As a result, the extent of the emphysema may vary, but it is characteristic that without treatment the disease runs a chronic course and that complete, spontaneous regression seems to be exceptional.

Probably, milder cases of congenital obstructive emphysema may occur without clinical signs in infancy, as several reported cases of obstructive emphysema in adults appear to have been of congenital origin (2, 21 and others).

At the Copenhagen County Hospital, Gentofte, we have observed 3 cases of localized emphysema of apparently congenital origin which will be reported here.

CASE 1. N.B.-C. A boy, aged  $2\frac{1}{2}$  months, was admitted in 1952 after a routine investi-

gation had revealed enlargement of the liver and spleen.

He had been born 3 weeks premature, but the birth history was otherwise normal. His birth weight was 3400 g and his length 50 cm. Prior to admission he had been in good health except that he failed to thrive.

On admission he was a thin, but otherwise normal-looking infant weighing 4100 g, with a slight, dry cough, but normal temperature. The liver and spleen were palpated as far as 3 to 4 cm below the costal border, but no other abnormalities were observed. Laboratory examinations, bone marrow examinations, and repeated differential blood counts failed to reveal any abnormalities. Moro's reaction and Wassermann reaction were negative. Throughout his stay in hospital, the boy seemed to be entirely unaffected, but he took his feedings only with reluctance and continued to thrive poorly. The first X-ray examination of the chest, taken 2 months after admission, disclosed a distinctly increased air content in the left hemithorax as well as a displacement of the heart and mediastinum to the right (Fig. 1). Repeated X-ray examinations during the remainder of his stay showed these findings almost unchanged. Since the pulmonary changes were interpreted as a possible left-sided pneumothorax, puncture of the left pleura in two sites was attempted about  $2\frac{1}{2}$  months after admission, but without result.

During the subsequent  $5\frac{1}{2}$  months the temperature was somewhat fluctuating, but apart from this there were no abnormal findings. When the temperature had been normal for one month, the patient was discharged. He was then almost 1 year old.

He was re-admitted at the age of 2 years 3 months, because he still failed to thrive. His height was now 84 cm and his weight 10 kg (average 89 cm/12.2 kg.). The liver and spleen were still palpable and X-ray examination showed an unmistakable emphysema of the left lung with flattening of the diaphragm, but without any cardiac displacement worth mentioning. Five days after admission, he developed an upper respiratory

Fig. 1. Age, 2 months. The left lung is enlarged and the mediastinum is displaced to the right.

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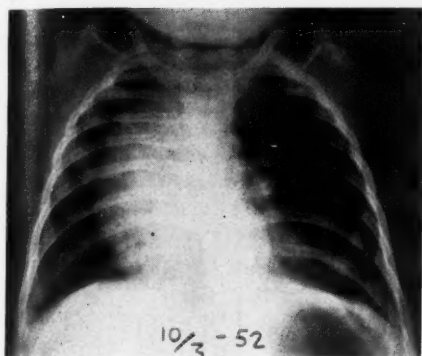


Fig. 1. Case 1, N. B.-C. Chest at 4  $\frac{1}{2}$  months of age. Severe emphysema of left upper lobe. Over the left diaphragm the shadow of the compressed lower lobe. Displacement of the heart and mediastinum to the right and depression of the diaphragm.

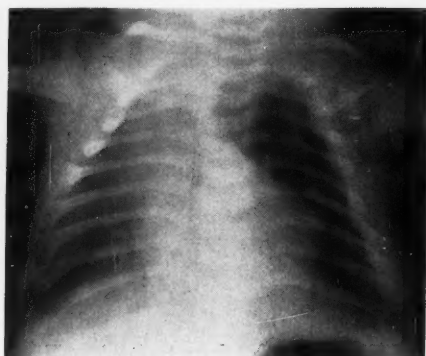


Fig. 2. Case 2, T.H., aged 1 month. Unmistakable emphysema of the left upper lobe with displacement of the great vessels to the right and flattening of both leaves of the diaphragm.

infection, and similar attacks continued during the subsequent 2 months. Bronchography showed basal displacement of the bronchial tree on the left side and filling of only the central 2 cm of the left upper lobe bronchus.

Left-sided thoracotomy disclosed severe emphysema of the upper lobe, so lobectomy was performed.

On examination of the specimen the emphysema was found to involve predominantly the anterior and apical-posterior segments, whereas the lingula was only moderately affected. No check-valve was demonstrable. Microscopic examination revealed—apart from the emphysema—inflammatory changes in the bronchi and bronchioles. No anomaly of the cartilaginous rings in the bronchial tree was found.

After discharge, the boy was as if transformed. He thrived well and was much more lively than previously. When last seen, 4 months after the operation, he was still making good progress.

**CASE 2.** T.H. A boy, 10 days old, was transferred in 1958 from a maternity department because of chondrodystrophy and a suspicion of heart disease. His birth was nor-

mal except for a slight asphyxia, but the following 3 days he had repeated attacks of cyanosis, and at one time he showed signs of cerebral hypoxia. During the subsequent days, he rallied, but was still cyanosed when crying.

On admission, he showed distinct retraction along the costal borders during respiration, but no cyanosis at rest. There was no elevation of temperature and no signs of respiratory infection. Physical examination showed his head to be strikingly large compared with his trunk, and hypertelorism, mild micrognathia and palatoschisis were noted. His chest was deformed, the sternum being short and very prominent, and he had a mild kyphoscoliosis of the dorsal spine. No auscultatory changes over the heart or lungs were heard. Limbs were very short, and both elbow joints were 45° short of full extension. His hands and feet were plump, and his spleen and liver were palpable below the costal borders. X-ray examination of the chest failed to show enlargement of the heart, but the great vessels were displaced to the right and there was emphysema of the left lung (Fig. 2). Similar, but less marked changes were seen on films taken at the age of 8 days. X-ray examination of the limbs

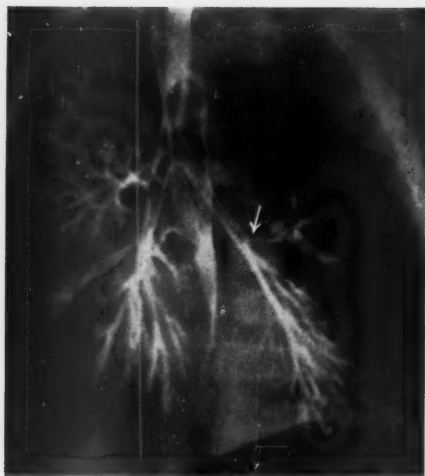


Fig. 3. Case 2, T.H., aged 5 months. Bronchography showing constriction and incomplete filling of a bronchus to the left upper lobe at its site of origin from the main bronchus. The other bronchial branches to the left lung are displaced basally.

showed changes corresponding to those seen in hypoplastic achondroplasia. Subluxation of both elbow joints was observed.

While in hospital, the patient had numerous attacks of cyanosis, especially when crying and taking food. He developed a cough, gradually with production of ample mucus. Repeated aspirations were of merely transient effect, and most of the time he had to be kept in an oxygen tent. Gradually, the condition improved so that, despite continued respiratory difficulty, he could be discharged to his home when a little over 3 months old.

When  $4\frac{1}{2}$  months old he was re-admitted because of upper respiratory infection of 8 days' duration. His condition was about the same as on his first admission. Bronchography revealed narrowing of the central part of the left upper lobe bronchus with incomplete peripheral filling, whereas the remaining branches of the bronchial tree were displaced basally (Fig. 3). The examination also aroused suspicion of a similar narrowing of a right middle lobe bronchus.

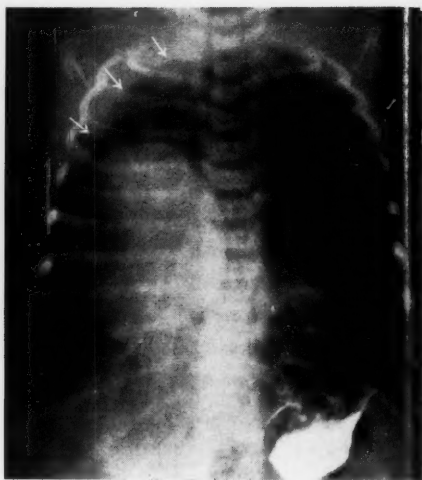


Fig. 4. Case 3, K.F., aged 2 days. Pronounced emphysema of the left upper lobe with compression atelectasis of the left lower lobe and right upper lobe. The arrows indicate the outlines of the left lung. Marked rotation of the heart and depression of the diaphragm.

Since repeated X-ray examinations showed increasing emphysema of the left upper lobe, left-sided thoracotomy was performed when the patient was  $5\frac{1}{2}$  months of age. The apico-pectoral part of the left upper lobe was found to be highly emphysematous and extended through the anterior mediastinum into the right hemithorax. Resection of the anterior and apical-posterior segments was performed. Postoperatively, the lung expanded well, but despite intermittent artificial ventilation through a tracheal tube, the patient's condition deteriorated and he died 6 days later.

Examination of the removed segments of lung showed the segmental bronchi to have been cut peripheral to the narrowing visualized by bronchography. On histological examination the lung tissue was emphysematous, but without pneumonic changes or specific inflammation. The bronchi and bronchioles were somewhat ectatic.

Autopsy revealed bilateral pneumothorax, with total collapse of both lungs, and cor pulmonale.

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Fig. 5. Case 3, K.F., aged 2 days. On a lateral view of the chest the left upper lobe may be seen forcing itself in front of the heart and elevating the sternum. Marked depression of the diaphragm.

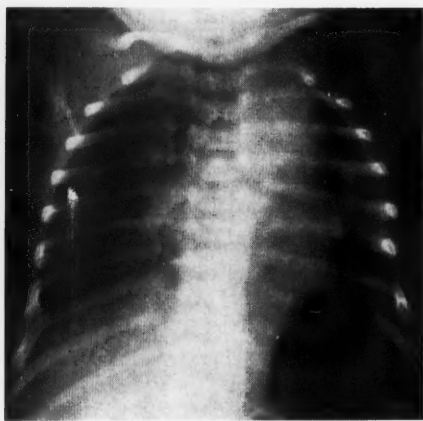


Fig. 6. Case 3, K.F., 11 days after resection of the left upper lobe. The drain has just been removed, and some pleural changes still remain at its former site in the apex, but the left lower lobe has expanded satisfactorily.

**CASE 3. K.F.** A boy, 1 day old, admitted in the spring of 1960. After delivery, which was uncomplicated, he had cried spontaneously. His birth weight was 3320 g and his length 51 cm. A few hours later, his breathing became laboured, and he developed cyanosis when crying.

On admission, auscultation revealed the respiratory sounds over the left hemithorax to be diminished, the heart displaced to the right, but no murmurs. The liver and spleen were distinctly palpable. E.C.G. indicated right axis deviation, but no other definite abnormalities. X-ray examination of the chest revealed severe emphysema of the left upper lobe, the heart being displaced backward and to the right (Figs. 4 and 5) and the left lung extending through the anterior mediastinum far into the right hemithorax. Moreover, there was an unmistakable depression and flattening of the diaphragm.

During the subsequent days, further exacerbation occurred. The infant turned

cyanotic upon the slightest exertion and required pure oxygen. Repeated X-ray examination showed increasing emphysema of the left lung, and at the age of 4 days thoracotomy was carried out as an emergency procedure. The emphysematous left upper lobe was removed and the bronchus cut at its site of origin from the main bronchus.

The postoperative course was uneventful. The compressed right upper lobe and left lower lobe expanded well, the cyanosis ceased completely, and 17 days after the operation the patient could be discharged (Fig. 6).

The removed lobe showed a short constriction of the main bronchus to the upper lobe, about 4 mm peripheral to where it had been severed. At this site, the lumen was less than 1 mm in diameter, while otherwise the bronchi were slightly ectatic. Microscopic examination showed the cartilage to be normally developed. The lung tissue was emphysematous, but without inflammatory changes.

### Discussion

According to previous reports, congenital emphysema has often given rise to great diagnostic difficulties, and many cases have been erroneously diagnosed at first. The differential diagnosis includes mainly (1) obstructive emphysema due to other causes, especially in pneumonia and aspiration of foreign bodies, (2) compensatory emphysema in pneumonia, atelectasis or hypoplasia of other lung segments, (3) pulmonary cysts, (4) pneumothorax, (5) diaphragmatic hernia, and (6) heart disease.

Most of these conditions are characterized by compromised function of parts of the lung. As a result, the clinical signs, dyspnoea, cough, and varying degrees of cyanosis, as well as the auscultatory findings are uncharacteristic, and radiography is of decisive diagnostic importance.

Congenital emphysema practically always involves the upper lobes or the right middle lobe. The lower lobe does not appear to have been involved except in one case (3). As another characteristic, it is nearly always lobar, whereas infectious obstructive emphysema generally manifests itself in the form of small, localized translucencies or else, especially in staphylococcal pneumonia, develops into cyst-like cavities, the so-called pneumatocoles. These changes may vary in appearance from day to day, and as a rule they soon yield to conservative treatment.

Aspirated foreign bodies, if not radio-paque and visible on a plain film, may usually be demonstrated by bronchography or bronchoscopy.

In cases of cysts, the diagnosis may usually be made by demonstrating the

wall of the cyst, and here tomography may be helpful. Incidentally, congenital emphysema may often run a more alarming course than the cysts (3).

Pneumothorax may generally be ruled out by demonstrating the scanty, but homogeneous pattern of the emphysematous lung. Most difficulties are encountered in cases of partial pneumothorax in which case screening and oblique exposures may be required.

Diaphragmatic hernia ought not to give rise to much diagnostic difficulty. At times, a contrast medium may have to be given orally in order to confirm the diagnosis.

Differentiation from compensatory emphysema is usually possible by screening the chest. In compensatory emphysema the heart and mediastinum become displaced away from the emphysematous side during inspiration, while in obstructive emphysema they are fixed or shifted toward the emphysema during inspiration. Furthermore, in obstructive emphysema there is usually depression and flattening of the diaphragm with reduced excursions.

Extended knowledge of congenital lobar emphysema and of the differential diagnostic problems should in most cases permit the correct diagnosis by comparing the results of repeated X-ray examinations with the history and clinical course.

A congenital aetiology in the present cases is indicated first and foremost by the apparent absence of primary pulmonary infection. It is characteristic, moreover, that in all three cases the signs appeared immediately after birth, in one only in the form of failure to thrive, but in the other two as dyspnoea and cyanosis. In all three cases the liver and spleen were found to be

palpable below the costal border, no doubt because both leaves of the diaphragm were depressed.

There is reason to stress the chronic course in the first two cases. Obstructive emphysema arising in the course of pneumonia usually disappears rapidly, within days or weeks, whereas congenital emphysema takes a chronic and not uncommonly progressive course in most of those cases which have been observed. A few cases with spontaneous, but incomplete remission have been reported (3, 11, 22).

Secondary respiratory infections are not uncommon in congenital emphysema, and the bronchitic changes found in the lobe removed from Case 1 do not militate against a congenital origin.

In the other two patients, the assumption of a congenital aetiology is further supported by the absence of inflammatory changes in the removed lobes and by the bronchographic and pathological findings respectively. Considering the chondrodysplasia in one of the cases, the possibility of localized chondromalacia of the bronchial tree being responsible for the bronchographic changes is present, although it was not demonstrated histologically.

### Treatment

In order to prevent deformity of the chest, secondary infections leading to acute exacerbation, and not least the risk of complications due to hypoxia, surgery is usually indicated as soon as possible. It should consist in removing the emphysematous segments of the lung after which the infants generally recover and develop normally. Only a few of the patients (11, 15, 20) appear to have remained

particularly prone to respiratory infections leading to transient emphysematous changes in the lungs.

In a few cases, attempts have been made to relieve the discomfort by direct puncture of the emphysematous segment of the lung. Kornbold & Baker (12) used this procedure successfully in two cases which remained free of recurrence for 6 months. In other cases, this treatment has been ineffective (9, 11, 15, 18), and in two it has even given rise to complicating tension pneumothorax with a fatal issue. As a result most authors now advise against this method.

### Summary

Three cases of congenital emphysema in infants are reported. All had clinical signs from birth and were treated by operation at the age of  $2\frac{1}{2}$  years,  $5\frac{1}{2}$  months, and 4 days respectively. Likely causes of the emphysema, in the form of narrowing of the respective bronchi, were found in only two of the patients, one of whom was also suffering from hypoplastic achondroplasia.

Aetiology, clinical features, and differential diagnosis are discussed, and the importance of early diagnosis and treatment is stressed.

Obstructive emphysema of congenital origin has to be considered as a differential diagnostic possibility, when an infant shows signs of respiratory disorder, consisting in dyspnoea, cough, and possibly cyanosis during the first months of life.

The diagnosis is based primarily upon the radiographic changes in conjunction with the history and clinical course.

As a rule, the course is chronic. In most cases the treatment is surgical, viz. resection of the emphysematous segments.

## References

1. BIERING, A.: Et tilfælde af lobært obstruktionsemfysem hos et spædbarn. *Nord Med*, 10: 1375-1376, 1941.
2. BRÜNNER, S. A. and POCK-STEEN, O. CH.: Localized emphysema. *Acta Radiol*, 53: 184-190, 1960.
3. CAFFEY, J.: On the natural regression of pulmonary cysts during early infancy. *Pediatrics*, 11: 48-64, 1953.
4. CASINELLI, J. F.: Hipoplasia de un bronquio pedicular y enfisema lobar agudo en un lactante. *Hoja Tisiol*, 4: 230-240, 365-376, 1944.
5. CONTRO, S., MILLER, R. A., WHITE, H. and POTTS, W. J.: Bronchial obstruction due to pulmonary artery anomalies. I. Vascular sling. *Circulation*, 17: 418-423, 1958.
6. DE BORD, R. A. and SIBILSKY, C. E.: Congenital obstructive emphysema treated by lobectomy. *Amer J Dis Child*, 88: 775-778, 1954.
7. FERGUSON, C. F. and NEUHAUSER, E. B. D.: Congenital absence of the lung (agenesis) and other anomalies of the tracheo-bronchial tree. *Amer J Roentgenol*, 52: 459-471, 1944.
8. FISCHER, H. W., POTTS, W. J. and HOLINGER P. H.: Lobar emphysema in infants and children. *J Pediat*, 41: 403-410, 1952.
9. GROSS, R. E. and LEWIS, J. E.: Defect of the anterior mediastinum. *Surg Gynec Obstet*, 80: 549-554, 1945.
10. HAMILTON, L. C. and GILLESPIE, R. W.: Congenital hypertrophic emphysema. *Amer J Roentgenol*, 80: 421-425, 1958.
11. HOLZEL, A., BENNETT, E. and VAUGHAN, B. F.: Congenital lobar emphysema. *Arch Dis Childh*, 31: 216-221, 1956.
12. KORNGOLD, H. W. and BAKER, J. M.: Non surgical treatment of unilobar obstructive emphysema of the newborn. *Pediatrics*, 14: 296-303, 1954.
13. LEAHY, L. J. and BUTSCH, W. L.: Surgical management of respiratory emergencies during the first weeks of life. *Arch Surg*, 59: 466-483, 1949.
14. LEWIS, J. E. and POTTS, W. J.: Obstructive emphysema with a defect of the anterior mediastinum. *J Thor Surg*, 21: 438-443, 1951.
15. NELSON, T. Y.: Tension emphysema in infants. *Arch Dis Childh*, 32: 38-41, 1957.
16. OVERSTREET, R. M.: Emphysema of a portion of the lung in the early months of life. *Amer J Dis Childh*, 57: 861-870, 1939.
17. POTTS, W. J., HOLINGER, P. H. and ROSENBLUM, A. H.: Anomalous left pulmonary artery causing obstruction to the right main bronchus. *JAMA*, 155: 1409-1411, 1954.
18. ROBERTSON, R. and JAMES, E. S.: Congenital lobar emphysema. *Pediatrics*, 8: 795-804, 1951.
19. SHAW, R. R.: Localized hypertrophic emphysema. *Pediatrics*, 9: 220-227, 1952.
20. SLOAN, H.: Lobar obstructive emphysema in infancy treated by lobectomy. *J Thor Surg*, 26: 1-20, 1953.
21. SWEET, R. H.: Case records of the Massachusetts general hospital. *New England J Med*, 242: 149-152, 1950.
22. TVETERÅS, E.: Congenitalt lobært emfysem. *Nord Med*, 59: 743-746, 1958.
23. VAN EPFS, E. F. and DAVIES, D. H.: Lobar emphysema. *Amer J Roentgenol*, 73: 375-386, 1955.
24. WHITE-JONES, R. H. and TEMPLE, L. J.: Congenital lobar emphysema. *Arch Dis Childh*, 29: 248-253, 1954.
25. WILLIAMS, M. H.: Localized pulmonary hypertrophic emphysema. *J Thor Surg*, 24: 522-529, 1952.

Received March 23, 1960

Accepted June 1, 1960

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## The Effect of Intramuscular Iron-dextran Complex<sup>1</sup> on Iron-deficiency Anaemia in Otherwise Ill Infants and Pre-school Children

by PHILIP LANZKOWSKY<sup>2</sup>

### Introduction

This paper describes a trial of intra-muscular iron-dextran complex in the treatment of iron-deficiency anaemia in ill Cape Coloured and African<sup>3</sup> infants and pre-school children attending the Out-patient Department of the Red Cross War Memorial Children's Hospital in Cape Town.

Parenteral therapy was especially chosen for treatment of these ill children since this was the only way of ensuring that they received the prescribed dose of iron.

It also eliminated frequent hospital visits and its attendant domestic difficulties.

Although oral iron is effective if given continuously for long periods, parents may fail for various reasons to administer it regularly and children may not readily accept the medication. The prevalence of acute diarrhoeal disorders (gastroenteritis) amongst under-privileged children is another reason for the employment of parenteral iron, since it cannot be certain to what extent oral iron is absorbed from the gut in the presence of diarrhoea.

Intravenously administered saccharated iron oxide is quite effective in the treatment of iron-deficiency anaemia. The technical difficulty of intravenous injections in small infants and the many recorded untoward reactions are the main disadvantages in its use.

Intramuscular iron-dextran complex, on the other hand, is safe, effective and easily administered. In infants and children with iron-deficiency anaemia a few workers have shown that iron-dextran complex results in a marked, rapid haematological and clinical improvement and that it is free from any undesirable side-effects (2, 6, 19).

<sup>1</sup> Imferon, Benger Laboratories Limited, England.

<sup>2</sup> Cecil John Adams Memorial Travelling Fellow, 1960, and recipient of a Dr. C. L. Herman Research Grant, University of Cape Town Staff Research Fund.

<sup>3</sup> The Cape Coloured people are of mixed ethnic origin and descended from various indigenous and imported groups: Slaves, Hottentots, Bushmen, Bantu and Europeans. The Africans, also referred to as "Bantu" or "Natives" consist of various tribes of indigenous groups of Southern Bantu. These people have a poor socio-economic status very much inferior to that of the White people. The African people occupy the lowest socio-economic position, whereas the Cape Coloured community is intermediate between the White and African. Both Cape Coloureds and Africans are ethnically "non-White" and their social conditions are similar to those of technically less advanced communities.

## Present Investigation

### Material

This investigation is based on a series of 125 consecutive Cape Coloured and African infants and pre-school children attending the Out-patient Department of the Red Cross War Memorial Children's Hospital in whom a diagnosis of iron-deficiency anaemia was made and who were treated with intramuscular iron-dextran complex. The patients were taken seriatim from those attending the out-patient department for various ailments. Acute diarrhoeal disorder (gastroenteritis) was the commonest illness amongst these infants and children. The majority of illnesses were acute and usually the duration was not more than a few days when the patient was brought to hospital. None presented primarily as cases of anaemia. The fact that these infants and children were out-patient cases does not imply that they were only mildly ill and not needing admission to hospital. There were many cases needing hospitalization but because of the shortage of accommodation they could not be admitted.

Any out-patient who showed clinical signs of dehydration or who had a blood dyscrasia, for example erythroblastosis foetalis or leukaemia, was excluded. The patients investigated had not received iron other than that present in their usual diet.

The mean weekly family income was £4.12 in the cases of Cape Coloured patients and £3.16 for African patients. This was determined by taking the weekly income of the head of the family in a sample of 50 consecutive patients in the two racial groups. Adopting the criteria of Bronte-Stewart *et al.* (4) and Moodie (13), these figures indicate that the Cape Coloured infants and children were from the "low income" group and the Africans from the "medium income" group in their respective sections of the community.

Of the Cape Coloured and African infants and children 50.3 % and 41.3 % respectively had been breast-fed for less than 3 months after birth. This is a much higher proportion than is found in healthy children in this

community (11, 12). In general, all these patients had a poor dietary history.

The diagnosis of iron-deficiency anaemia was based on the level of haemoglobin and the morphology of the red blood corpuscles on the blood smear. The critical level of haemoglobin at or below which anaemia was considered to be present and iron medication administered was arbitrarily fixed at 0.5 g % below the lowest figure accepted by Wintrobe (18) as normal for each age group.

Concurrently with the necessary treatment for the presenting illness these infants and children were treated for iron-deficiency anaemia with iron-dextran complex given by deep intramuscular injection. The amount of iron-dextran complex was calculated according to the original haemoglobin level and the body weight<sup>1</sup> and varied from 100 to 400 mg (2-8 ml).

### Methods

Blood for haemoglobin was taken from the heel prick of infants and the thumb of older children, using a triangular cutting needle. Free unrestricted flow without necessity for external pressure of any kind was obtained in all cases.

Capillary blood, 0.02 ml, was pipetted into 5 ml of 0.04 % ammonia in water and the haemoglobin level read by the oxyhaemoglobin method using a Klett-Summerson Colorimeter previously calibrated for the purpose against standard haemin and cyanmethaemoglobin solutions. Blood smears were stained by May-Grünwald Giemsa method.

### Results

Haemoglobin estimations were made immediately before commencement of iron medication, and at three weeks and six weeks thereafter.

<sup>1</sup> The formula used was  $9W + \frac{W}{6} (100 - \text{Hb } \%)$   
= mg of iron,

where W = Weight of patient in pounds

Hb % = Observed haemoglobin percentage  
(as recommended by the manufacturers of iron-dextran complex).



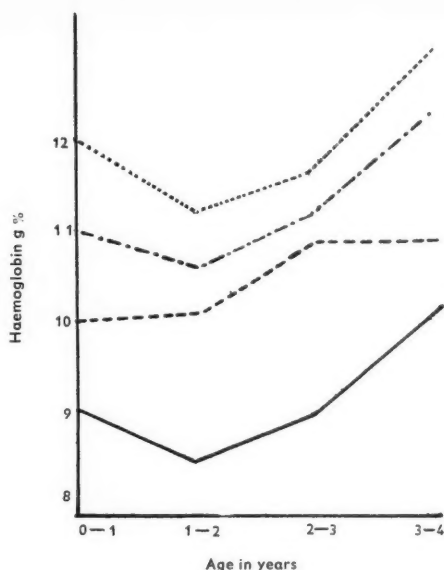


Fig. 1. Mean haemoglobin levels in 125 ill Cape Coloured and African infants and pre-school children before and after treatment with intramuscular iron-dextran complex.

Key: Initial Hb. —, after 3 weeks — —, after 6 weeks — · — ·, Normal value (Wintrobe) · · · ·

The mean haemoglobin levels before and after treatment with iron are shown in Figure 1 and Table 1. The mean haemoglobin levels before treatment ranged from 8.91 g % to 9.61 g % in the various age groups, at 3 weeks after the injection of iron the mean haemoglobin levels were found to range between 10.12 g % and 10.80 g % and at 6 weeks after injection the mean haemoglobin levels had risen further to ranges between 10.75 g % and 12.0 g %. The morphology of the red blood corpuscles on the blood smear, prior to treatment, showed all gradations of an iron-deficiency pattern.

None of the children showed severe untoward reactions after iron administra-

tion. Occasionally fever lasting 24 to 48 hours was observed and less commonly localized staining of the skin occurred when there was leakage beneath the skin if the iron was not given deep enough into the muscle, or because "streaking" occurred along the path of withdrawal of the needle. This was found to be of no consequence. There was no local tenderness, redness or swelling. The pain during administration, as judged by the reactions of the children, did not appear to be more than that occurring with any other intramuscular injection.

It is clear that, in spite of the presence of an infection, there was a statistically significant increase in haemoglobin levels.

TABLE 1. *Haemoglobin levels in 125 ill infants and children treated with intramuscular iron-dextran complex*

Age (yrs.)	Pre-treatment		Post-treatment				Significance P
			3 weeks		6 weeks		
	Number of cases	Mean Hb. g. %	Number of cases	Mean Hb. g. %	Number of cases	Mean Hb. g. %	
First	68	8.91	62	10.12	28	11.01	< 0.001
1-2	34	8.45	33	10.20	13	10.75	< 0.001
2-3	15	8.83	10	10.76	6	11.23	< 0.001
3-4	8	9.61	5	10.80	7	12.00	< 0.01
Total	125		110		54		

(Significances calculated between pre-treatment & 6 wks. post-treatment levels.)

It is also apparent that, even under the easiest conditions that could be devised, there was a very considerable default list. Rather less than half of the children returned for follow-up haemoglobin estimations on the second scheduled date six weeks after the intramuscular injection of iron-dextran complex. This impairs full assessment of results but the number of patients who did attend for follow-up was sufficient to indicate that this form of treatment in sick children is undoubtedly beneficial. An approximate general rise of 2 g % of haemoglobin in 6 weeks is shown. The younger children responded better than the older ones. This was probably related to the haemoglobin level, since the response to iron, generally speaking, varies inversely with the initial haemoglobin level.

The beneficial effects of intramuscular iron-dextran complex on the general well-being of the children was striking. An improvement in activity, colour and appetite was noted in virtually all the patients and in many patients this was ap-

parent within 2 to 3 days after treatment was started. Although this may have been partly due to concurrent therapy for presenting illness, parents commented on the better condition compared with the pre-illness state. Confirmation of the increase in appetite was given by the increase in weight which was a common and striking feature of the patients' records.

The results are presented here as evidence that intramuscular iron-dextran complex can significantly raise the haemoglobin level in infants and children who have iron-deficiency anaemia and a variety of associated illnesses, of all degrees of severity, and of largely undetermined duration.

This treatment has also been applied to some in-patients, but the situation was usually complicated in these by the urgent need for fluid replacement or blood transfusion which makes an assessment of the results of treatment with intramuscular iron-dextran complex difficult. For this reason the results of treating the in-patients with iron have not been analyzed.

### Discussion

After intramuscular administration of iron-dextran complex, the rate of recovery from iron-deficiency anaemia depends on several factors of which the most important are the presence and severity of the associated infection, the type of tissue into which the iron is injected, the mobilization of the limb and the initial degree of iron shortage.

The anaemic sick infants and children in the present investigation responded well to intramuscular iron-dextran complex. The presence of acute infection, and of some infections which were of longer duration, did not prevent a significant increase in haemoglobin level. The duration of what has been called "acute infection" cannot be accurately and definitely determined but generally was not more than a few days. Although they were all out-patients they were not all mildly ill. Indeed, an appreciable number of the patients were severely ill.

Absorption of intramuscular iron-dextran complex may be diminished if a proportion of the dose becomes trapped in the subcutaneous fat (8). The evidence of this is gross skin-staining after injections. In obese subjects particularly, some of the dose may enter fatty tissue (16). This is really due to faulty injection technique. Since the majority of the present subjects were undernourished, many of them grossly so, the factor of obesity need not be discussed any further.

Another factor influencing absorption is the mobilization of the limb following intramuscular injection. Grimes & Hutt (8) stated that four of their patients were confined to bed at the time of the injection, and the least mobile showed the lowest

eventual clearance. They concluded that exercise of the limb after injection increases absorption, and that in obese immobile patients one should administer a dose slightly higher than the calculated requirement of iron.

All the subjects of the present study were out-patients. It is reasonable to assume that all those over one year of age were more than ordinarily active since, for example, their economic circumstances would force them to walk where children in better circumstances are transported.

In the investigation done by Wallerstein & Hoag (17) the magnitude of the haemoglobin response varied with the degree of anaemia. The haemoglobin level in some of the children with severe anaemia increased by 4 % per day during the first week of treatment while others with less marked anaemia showed little or no measurable rise during that time. In the present investigation as well, the response to intramuscular iron-dextran appeared to vary inversely with the initial haemoglobin level.

It was the author's impression that improvement in appetite and diminished irritability after intramuscular iron-dextran complex appeared before any rise in haemoglobin level was demonstrable. This rapid response was also observed by Yu *et al.* (19). Beutler's (3) view is that part of the symptoms of iron-deficiency anaemia may be related to deprivation of tissue iron enzyme and not chiefly to the anaemia. He deduced this by showing a marked decrease in the cytochrome c content of the liver and kidneys in iron-deficient rats. This decrease greatly exceeded the slight decrease in haemoglobin concentration.

The total calculated dose of iron-dextran complex in this investigation was given at the initial consultation. This was done in order to reduce the number of hospital visits which in a poor community entails considerable financial and domestic hardship. Despite these very large doses no severe untoward local or systemic effects were observed. It should not, however, be assumed that there is no limit to the amount which can be given.

Prior to the commencement of administration of parenteral iron consideration was given to the possibility of producing haemosiderosis or haemachromatosis. Since there is no effective mechanism for iron excretion, injected iron is almost entirely retained and excessive therapy might lead to iron overload. Yu *et al.* (19) have stated that humans cannot excrete more than 1 mg of iron in 24 hours. There is a wide margin of safety, however, and at least 1 g of intramuscular iron-dextran complex may be given to infants with impunity (17). The amount of iron needed to produce haemosiderosis or haemachromatosis is approximately 25 g. As the maximum therapeutic dose in the adult is 5 g, there is a five-fold safety factor (5). In infants receiving 100 mg to 400 mg the safety factor is probably much higher. One would not expect this to be a problem provided that the proper dosage is used and a safe maximum is not exceeded. No evidence of a detrimental effect in the children of the present investigation has so far appeared. Nevertheless, it must be strongly emphasized that failure of a patient to respond to the proper intramuscular dose of iron is never an indication for giving more iron.

This is important especially in the light

of recent research work which has shown that iron-dextran complex in massive doses may result in excessive deposition of iron in the interstitial cells of the testes and testicular atrophy in mice (14) and that it may induce sarcoma at the site of injection in rats and mice (9, 10, 15). The dosage of iron-dextran complex used by Richmond (15) was massive—some 200- to 300-fold greater than the therapeutic dose and that used by Haddow & Horning (10) represented in relation to body iron, a swamping dose, both locally and systemically. The "clinical dose" for mice would be only 1/100th or 1/150th of that used in Haddow & Horning's experiment (10). In the lower dose used by Richmond (15), the incidence of tumours dropped sharply to become comparable with, or even lower than, published figures for tumours obtained in a similar manner by repeated injections in rats and mice of glucose, fructose, arachis oil, and many other innocuous materials (7).

Apart from the important dosage factor, repeated trauma, muscle necrosis and chronic inflammatory changes caused by repeated injection into the unstable subcutaneous tissues of rats and mice can lead to the production of sarcomata (7). Sarcomata following injection of iron-dextran complex have been produced with any degree of frequency only in rats and mice. Rat connective-tissue cells are notoriously susceptible in responding with sarcomas to injection of the most diverse agents (1). Other species which have received massive over-doses of iron-dextran, for example, the rabbit and guinea pig, did not develop tumours (10).

An assessment of the clinical significance and implication of these observa-

tions is not possible at present and will require an elaborate study of the many factors involved.

### Summary

1. One hundred and twenty-five ill infants and children who were found to have iron-deficiency anaemia were treated with intramuscular iron-dextran complex with doses varying from 100 to 400 mg, concurrent with treatment for the presenting illness.

2. The mean haemoglobin levels before treatment ranged from 8.91 g % to 9.61 g % in the various age groups; at 3 weeks after the injection of iron the mean haemoglobin levels were found to range between 10.12 g % and 10.80 g % and at 6 weeks after injection the mean haemoglobin levels had risen further to range between 10.75 g % and 12.0 g %. There was thus an approximate general rise of 2 g % of haemoglobin in 6 weeks. Clinically the beneficial effects were quite striking, and occurred often within days.

3. The results of this investigation indi-

cate that intramuscular iron-dextran complex can significantly raise the haemoglobin level in infants and children who have iron-deficiency anaemia with a variety of associated illnesses, of all degrees of severity, and of largely undetermined duration. In addition, iron-dextran complex was found to be safe and easy to administer.

4. The factors influencing rate of recovery from iron-deficiency anaemia after intramuscular iron-dextran complex, the theoretical dangers of iron overload and the possible production of sarcomata in iron-injected tissue are discussed.

### Acknowledgements

I am indebted to Professor F. J. Ford, head of the Department of Child Health, University of Cape Town, for his constant guidance, advice and constructive criticism; Dr. H. Myers for his help in the presentation of this work; Dr. J. F. W. Mostert, Medical Superintendent, Red Cross War Memorial Children's Hospital, for permission to publish this work and Dr. D. McKenzie for providing me with the facilities of his pathology laboratory.

### References

1. ANNOTATION: Ten Years with Tumour Viruses. *Lancet*, 2: 227, 1957.
2. BARTLETT, W. H. and BEATTY, E. C.: The treatment of iron-deficiency anaemia in children with iron-dextran. *Amer J Dis Child*, 94: 662, 1957.
3. BEUTLER, E.: Iron enzymes in iron deficiency. I. Cytochrome C. *Amer J Med Sci*, 234: 517, 1957.
4. BRONTE-STEWART, B., KEYS, A., BROCK, J. F., MOODIE, A. D., KEYS, M. H. and ANTONIS, A.: Serum cholesterol, diet and coronary heart disease: an interracial survey in the Cape Peninsula. *Lancet*, 2: 1103, 1955.
5. COLEMAN, D. H., STEVENS, A. R. Jr. and FINCH, C. A.: Treatment of iron deficiency anaemia. *Blood*, 10: 567, 1955.
6. GAISFORD, W. and JENNISON, R. F.: Intramuscular iron in infancy. *Brit Med J*, 2: 700, 1955.
7. GOLBERG, L.: "Imferon" Letter to editor. *Brit Med J*, 1: 958, 1960.
8. GRIMES, A. J. and HUTT, M. S. R.: Metabolism of 59 Fe-dextran complex in human subjects. *Brit Med J*, 2: 1074, 1957.
9. HADDOW, A. (1958): (Quoted by Richmond, H. G.). Personal communication.
10. HADDOW, A. and HORNING, E. S.: On the carcinogenicity of an iron-dextran complex. *J Nat Cancer Inst*, 24: 109, 1960.
11. LANZKOWSKY, P.: Iron-deficiency anaemia in infants and pre-school children in three racial groups in Cape Town. M.D. Thesis, University of Cape Town, 1959.

12. — Mean haematological values in healthy infants and pre-school children in Cape Town. *S Afr Med J*, 34: 469, 1960.
13. MOODIE, A. D.: Personal communication, 1959.
14. NISSIM, J. A.: Deposition of iron in testes after administration of iron-dextran complex. *Lancet*, 1: 701, 1955.
15. RICHMOND, H. G.: Induction of sarcoma in the rat by iron-dextran complex. *Brit Med J*, 1: 947, 1959.
16. SCOTT, J. M.: Intramuscular iron therapy in anaemia of pregnancy; 2-year survey. *Brit Med J*, 2: 635, 1956.
17. WALLERSTEIN, R. O. and HOAG, M. S.: Treatment with iron-dextran of iron deficiency anaemia in children. Paper read before the 10th Clinical Meeting of the *Amer Med Ass, Seattle*, Nov. 3<sup>rd</sup>, 1956.
18. WINTROBE, M. M.: Normal values for number of erythrocytes, quantity of haemoglobin and volume of packed red cells. *Clinical Haematology*, 4th ed. Philadelphia, Lea and Febiger, 1956, p. 100.
19. YU, W. L., MANONMANI, K. and WATSON, R. J.: Comparison of intramuscular iron-dextran and oral ferrous sulfate in the treatment of iron-deficiency anaemia. *J Pediatr*, 54: 50, 1959.

Received April 5, 1960

Accepted May 27, 1960

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## A Study of Group A Hemolytic Streptococcus Carriers Among School Children

### Part II. Significance of the Findings<sup>1</sup>

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E. ORSSAUD and P. VIROLLEAU

#### Discussion

The results which we obtained in the course of the study are of two kinds: those which are of bacteriological interest, and those which chiefly concern the epidemiology of the hemolytic streptococcus in healthy subjects.

#### 1—Bacteriological study

One of the aims of our study was to develop a technique of taking throat swabs and of their subsequent culture which would be as sensitive as possible and suited to the needs of our epidemiological survey.

Between the taking of the throat-swab and the grouping of the organism, a number of difficulties and possible causes of error may arise. These may affect the following areas: the material used for the throat swab and the way in which it is taken; the transport of the swab; isolation (with or without the use of a selective medium); the reading of the blood-agar

plates; finally, the culture medium used for the re-seeding of suspect colonies.

A—*Material, technique and transport of swabs.* In the course of meetings of the Committee on Acute Respiratory Diseases in 1946 and 1948 (3), Wannamaker *et al.* (25) showed that isolation should be done on the spot so as to avoid the risk of the throat swab drying up. Rubbo & Benjamin (21) have demonstrated that cotton wool rapidly kills the organism and that the survival of the streptococcus in the throat swab will increase from 2 days to 10 if the carded cotton used is dipped in clotted serum. In a general way, all the authors have advised against the transport of the throat swab as such in a dry state; this has led Pike (14), Wannamaker (24), Holmes (9) and Werner & coll. (26) to use selective media for the detection of healthy carriers; the same has been the case for the detection of carriers during streptococcal infections or in rheumatic fever (Feldman & Harmon (6)).

For our part, we endeavoured to eliminate these causes of errors by performing all the initial manipulations on the spot:

<sup>1</sup> Part I was published in the November issue of Acta Pædiatrica 1960.

TABLE 8.

Authors Date	No. of swabbings or of A found	% of A found	
		without medium	with selec- tive medium
Pike—1945	131	16	51
Wannamaker & coll. 1948	339 A	31.3	61.9
Feldman and Harmon, 1952	103 A	20	26
Holmes—1956	89 subjects	68.4	81.6
Werner—1958	78 A	50	69

(1) when the swab was being studied without the use of a selective medium, the blood-agar plates were brought to the place where throat swabbing sessions were being held and isolation was performed then and there. (2) when we used selective media, the tubes were brought to the place of swabbing and the throat swab was immediately introduced into the fluid or semi-solid medium.

B—*Direct isolation and isolation after passage through a selective medium.* Pike (13) demonstrated that crystal violet and sodium azide added to broth favour the growth of hemolytic streptococci while inhibiting that of associated germs. The subsequent studies of Wannamaker (24), Feldman & Harmon (6), Holmes (9), and Werner (26) confirmed his conclusions. Holmes (9) wishing to devise a suitable transport medium, modified the concentrations of sodium azide and crystal violet given by Pike and added agar-agar, to render it semi-solid. The comparative results obtained by these authors are summed up in Table 8.

On the other hand, these authors were able to elucidate a number of points concerning isolation proper, whether direct isolation or isolation after passage through a selective medium. Thus, Wannamaker &

coll. demonstrated (25) that more positive swabs are found in healthy carriers if as many suspect colonies as possible are taken from a single plate and if several plates are seeded with the same throat-swab.

Fry (8) and Holmes (10) have emphasized the favourable effect of anaerobiosis, and Werner & coll. of mass seeding (26).

Under the working conditions of our laboratory it was not possible in 1957–1958 to seed several plates with the same throat-swab. An attempt was made to re-seed as many suspect colonies as possible and we made a special point of studying and developing an efficacious selective medium. This led to the study of 3 media all of which were based on a meat extract broth buffered at pH 7.8; they differed in their concentration of crystal violet and sodium azide.

During the year 1957–1958, 2 media were thus investigated comparatively in direct isolation on blood-agar. The negative results obtained with the first of these media (Technique 2) soon led to the abandonment of these concentrations of sodium azide and crystal violet and to the adoption of a medium rather similar to that proposed by Holmes.

This technique enabled us to demonstrate up to 10 times as many group A strepto-

TABLE 9-11. *Direct smears and fluid medium adapted from Pike.*

TABLE 9.

	Basic medium	Concentration and added products	No. of swabs	No. of A	% of A
Technique 1	—	—	215	7	3.2
Technique 2	Peptoned, glucosed meat extract broth pH 7.8	NaN <sub>3</sub> 1/400,000 Crystal violet 1/500 Defibrinated blood 3/100	193	0	0

TABLE 10.

	Basic medium	Concentration and added products	No. of swabs	No. of A	% of A
Technique 1	—	—	663	20	3.1
Technique 3	—	NaN <sub>3</sub> 1/1,000,000 Crystal violet 1/16,000 Agar-agar 15/1000	570	125	21.9

TABLE 11.

	Basic medium	Concentration and added products	No. of swabs	No. of A	% of A
Technique 3	—	NaN <sub>3</sub> 1/1,000,000 Crystal violet 1/16,000	191	21	10.9
Technique 4	—	NaN <sub>3</sub> 1/1,000,000 Crystal violet 1/16,000 Autolysate 3/1000	237	38	16

cocci as with direct isolation on a blood-agar plate. Contrary to the opinion of most authors who have used Pike's medium (especially Feldman (66)), we have not observed that our semisolid medium derived from that of Holmes particularly favoured streptococci of Groups B, C and G. On the contrary, as regards Group C and G streptococci, we could not obtain an increase as great as that of Group A.

During the year 1958 it was this medium derived from that of Holmes, which

was used for purposes of comparison. In the 3rd medium used, the concentrations of sodium azide and crystal violet were not modified, the agar-agar was omitted so as to facilitate a better dispersion of the organisms and some barm autolysate was added to increase the growth of the streptococcus even more.

This technique did not improve our results as strikingly as did Technique 3 when compared with direct seeding; nevertheless, this medium (Technique 4) enabled

TABLE 12.

No. of carriers	13	25	20	17	15	11	15	13	14	10	9	11	7	18	16	9	9	9	10
No. of children	85	84	85	87	85	71	84	87	84	86	87	85	87	85	89	85	88	86	86

$\chi^2 = 34.5$  with  $V = 18$  degree of freedom  $P = 0.01$ .

TABLE 13.

Survey	Year	Number of cases	Maximum %
Bourn	1933	2 812	No relationship
Rantz	1941	643	Winter and early spring
Riley	1953-1955	175	No relationship
Saslaw	1953	343	May
	1953-1954	417	Febr./Apr. May
	1954-1955	333	Febr./March
	1954-1955		
	Special study		March
Cornfeld	1955-1956		Jan./April, May
	School W	468	
	School B	456	Jan., May
	1956-1957		
	School W	454	January
	School B	385	April

us to further increase the rate of our carriers in a marked and statistically significant way. Tables 9, 10 and 11 sum up the results obtained.

We believe, therefore, that, for the time being, medium 4 is the most sensitive and the most suitable for epidemiological studies of the type that we have undertaken.

#### 11—Epidemiological study

*A—Variations in the percentage of carriers.* We have seen (Fig. 1. cf. Part I) that the percentage of carriers varies during the course of the year. This variation is statistically significant, i.e. within the limits of precision of the method used, it is not due to chance fluctuations among a population with a constant rate of carriers (Table 12).

Most of the authors who have performed

regular throat swabbings have noted similar variations which they were naturally inclined to ascribe to seasonal factors (Table 13).

These studies are difficult to compare because of differences in the techniques used. It is, however, generally agreed that streptococcal morbidity reaches a peak at the end of winter and in early spring. It has been seen that most authors consider this to be true for carriers as well. Our own observations would seem to lead to the same conclusion. It should be pointed out, however, that several theoretical conditions must be fulfilled before variations in carrier percentages can be linked in an unquestionable way with seasonal influences. The variation around the average must not be due to chance. We have seen that this condition has been fulfilled in the case of our results. The curve

of seasonal variations should reproduce itself regularly from year to year and for several years running. The latter condition is much more difficult to abide by. It is, in fact, impossible to consider following a population for some ten years with the sole purpose of verifying the existence of an annual peak; in addition, the changes likely to occur during that period in the population studied would restrict the meaning of the observations; lastly, changes in microbial diffusion over the years would make the conclusions reached only relative.

However, the remarkable agreement of several of the studies referred to above added to our own would give us reason to believe that there is an annual peak of the percentage of carriers in early spring. It should be noted in this connection that the four concurring studies (Rantz, (19), Saslow (23), Cornfeld (4) and ourselves) have borne on different populations followed up at different periods (1941, 1953 to 1958), in different regions (all situated, however, in the temperate regions of the northern hemisphere). This divergency of conditions to our mind makes the similarity of the results all the more significant.

A third condition is the demonstration of a direct relationship between the cyclical variation and a measurable seasonal factor. The definition of a season is, of course, difficult to express in figures although it is current practice to note a change of season objectively. We attempted to verify the existence of a correlation between the percentage of carriers and certain meteorological factors. We found that there was no detectable relationship between this percentage and the average temperature, rainfall, barometric

pressure, or the total number of hours of sunlight.

There apparently exists a correlation with average humidity although of a type that would seem contrary to generally accepted ideas. Indeed, our percentage of carriers varies considerably in inverse ratio to the percentage of average humidity. We believe, however, that this relationship is not a real one and that it is due to the fact that in the region investigated, the relative humidity shows a marked decrease from the month of March onwards.

The question of seasonal variations and of the factors that bring them about is still open. Strictly standardized observations are required before it may be regarded as having been finally resolved.

*B—The problem of the carrier factor.* When it is noted, as mentioned above, that the carriers can be fairly easily divided into two groups: casual carriers and prolonged carriers, the question naturally arises whether this difference of behaviour is due to a specific factor. The problem may be approached in different ways. In this study, we have attempted to solve it by means of the statistical method. If no special factor is involved influencing the distribution of carriers, the latter is purely a matter of chance. It can then be compared to a dealing out of a pack of playing cards, where the chances any subject has of receiving the "streptococcus card" can be calculated for each deal, since we know each time the probability of distribution of these streptococcus cards.<sup>1</sup>

<sup>1</sup> This probability depends on the percentage of carriers following each swabbing, i.e. it varies from one deal to another and must be recalculated each time.

TABLE 14.

	Children with tonsils		Children tonsillectomized		Total
	No.	%	No.	%	
Non carrier	13	52	12	48	25
Carrier 1 to 4 times	19	70	8	30	27
Carrier more than 4 times	17	89	2	11	19
Total	49		22		71

$\chi^2 = 7.2$  with  $V = 2$  degrees of freedom.  $P < 0.05$ .

In these circumstances, the theoretical cumulative percentage of carrier subjects can also be determined. After a certain number of deals it should, of course, tend to 100 %, the chances being equal for all the subjects to receive the "streptococcus card" at a given time.

To bring this point out more clearly, we tried to select as homogeneous a group as possible out of our total population. Statistical calculation having shown that absence from more than 5 swabbings created a factor of heterogeneity, we discounted all subjects who had been absent 5 times or more during the study. We were thus left with a more restricted but a more homogeneous sampling of 89 children.

Figure 6 (cf. Part I) opposes the theoretical curve calculated in this way and the real curve which we have observed. It will be easily seen that the latter rises rapidly, at first, but then stays at a level much below the theoretical maximum. It is therefore permissible to infer that the distribution of the "streptococcus card" is not due to chance and that consequently there is a systematic factor connected either with the existence, in the prolonged carrier, of a special facility for acquiring the organism, or to the existence, in the habitual

non carrier, of a special defence against the same organism.

The real nature of this factor cannot, of course be ascertained by the same method; this can only be done by biological investigation.

*C—Biological and sociological factors connected with the carrier state.* Thinking our belief in the existence of a carrier factor to be justifiable, we attempted, by means of a medico-social questionnaire of each of the children observed, to determine the correlations that might exist between the carrier state and the physiological and environmental conditions of the subject.

The investigations were made in the same group of subjects as above, and for statistical reasons we considered as prolonged carriers the children whose throat swabs were A positive 5 times or more during the survey.

We were thus able to demonstrate 3 definite correlations:

(1) Tonsillectomized subjects are less often prolonged carriers than those who still have their tonsils (Table 14). This idea has already been generally accepted and with the exception of Quinn & Riley (17) all the



TABLE 15.

	No running water		Running water		Total
	No.	%	No.	%	
Non carrier	1	4	24	96	25
Carrier 1 to 4 times	7 <sup>1</sup>	26	20 <sup>1</sup>	74	27 <sup>1</sup>
Carrier more than 4 times	5 <sup>12</sup>		14 <sup>34</sup>		19 <sup>46</sup>
Total	13		58		71

$\chi^2 = 5.4$  with  $V = 1$  degree of freedom.  $P = 0.02$ .

TABLE 16.

	Brown eyes		Blue eyes		Total
	No.	%	No.	%	
Non carrier	7	28	18	72	25
Carrier 1 to 4 times	17	63	10	37	27
Carrier more than 4 times	12	63	7	37	19
Total	36		35		71

$\chi^2 = 8.0$  with  $V = 2$  degree of freedom.  $P = 0.02$ .

authors who have undertaken a similar study have reported such a correlation (5, 7, 11, 15, 18, 27).

(2) The environmental health conditions of the carrier are also a well-known factor. We have found a strong correlation between the prolonged carrier state and a particular factor of hygiene namely the absence of running water in the dwelling-house (Table 15).

It is evident that the absence of running water in an apartment nowadays indicates a very low standard of sanitation, especially in an urban environment. It is, however, interesting to note that we have recorded no other correlations between the prolonged carrier state and other criteria of hygiene such as those connected with the situation of the dwelling-house (lighting, ventilation, dampness) amenities

such as heating, gas, electricity, or the conditions of accommodation; as regards the latter point, it is to be reported that we have found no correlation between the carrier state and overcrowded lodgings.

(3) As regards genetic factors, it is generally asserted that heredity plays a part in certain complications of streptococcal infection; in particular rheumatic fever. But, although widely accepted, this view has not been established on the strength of any very sound observations, with the exception of some recent studies which report that rheumatic subjects belong to certain blood groups more often than controls.

Be that as it may, the predisposition to streptococcal infection and to its complications (especially rheumatic fever) is a very different thing from the predisposition

TABLE 17.

	Children with tonsils		Children tonsillectomized		Total
	No.	%	No.	%	
Running water	36	62	22	38	58
No running water	13	100	0	0	13
Total	49		22		71

$\chi^2 = 7.07$  with  $V = 1$  degree of freedom.  $P < 0.01$ .

TABLE 18.

	Brown eyes		Blue eyes		Total
	No.	%	No.	%	
Children with tonsils	27	55	22	45	49
Children tonsillectomized	9	41	13	59	22
Total	36		35		71

TABLE 19.

	Brown eyes		Blue eyes		Total
	No.	%	No.	%	
Running water	30	52	28	48	58
No running water	6	46	7	54	13
Total	36		35		71

to being a transient or chronic carrier which is what interests us at the moment. Our study demonstrates a definite correlation between the carrier state and a genetically controlled factor viz. the dark colour of the iris (Table 16). To our knowledge this is the first time that a correlation of this kind has been reported in this field. It has been verified that this correlation is neither associated with a common ethnic origin nor with the fact of belonging to the same residential area.

There is a partial correlation between the presence of running water in the apartment and tonsillectomy (Table 17). On the contrary, the correlations between the colour of the iris and tonsillectomy (Table 18) or the running water (Table 19) are unconnected: they are therefore independently significant.

For other physiological or sociological factors we have found slight connections which do not allow of unqualified conclusions. A new survey is in progress, one

of the aims of which is precisely to attempt to bring out the probability of these correlations more clearly.<sup>1</sup>

### 111—*The limits of interpretation*

We have raised the question of the extent to which the considerable inadequacies of our technique of bacterial research, as shown by the error calculation set forth above, might limit the scope of the conclusions which we believe to have reached, especially as regards the existence of prolonged carriers, for this problem seems to be in the forefront nowadays.

It is evident that since the results of swabbing, performed in identical conditions, vary markedly with the technique used, questions of technique greatly modify the value that can be ascribed to them.

The manipulations which lead to the final identification of a Group A streptococcus in a throat swab are many, and

their importance for the reliability of the ultimate result varies. We have set forth above, in the course of the discussion of our technical results, several points that the experience of other authors and ourselves permitted us to elucidate, but it must be admitted that a searching methodological study has not yet been made.

It can be seen that the technique on the results of which our figures are based (Technique 3) fails us once every 3 times (34.5 % of failures). No categorical value can therefore be attached to a single negative swab either for concluding that a child is not a carrier or for asserting that he has ceased to be one.

On the other hand, when a subject hitherto negative happens to present a positive swab at a given time, we are unable to claim that this is a recent acquisition of the organism, since Table 7 clearly brings out the fact that the same strain can be found in a subject after a long period of apparent absence.

The imperfections of the method lead us therefore to results falsified by default, on the one hand, since we regard as negative the subjects who have one chance in three of being carriers, in fact, and by excess, on the other, if we consider as an acquisition the reappearance in a subject of a strain which had been lost for a time due to an inadequate technique.

It is, of course, impossible to guard against the first of these errors. The second can, however, be eliminated by considering as an acquisition in a given subject only the appearance of a new type of strain. As we had no regular typing of all our strains, we have been unable to use this method, and, this being so, we have decided to base our argument on the cumulative incidence

<sup>1</sup> The following correlations with the state of being a "prolonged carrier" were studied statistically: Hair; Eyes; Complexion; Twins; Congenital malformations; Age of the father; Age of the mother; Siblings; Weight; Height; Puberty; Personal cleanliness; General condition; Cuti-reaction—positive due to BCG; Streptococcal disease; Surgical operations; Hospitalisation; Kind of dwelling-place; Floor inhabited; Exposure to sunshine; Ventilation; Humidity; Heating; W.C.; Water supply; Hygiene conditions; Electricity; No of persons living at home; No of rooms; Presence of the father at home; Allergy in the father; Streptococcal disease in the father; Father's profession; Father's conditions of work; Conditions of hygiene of the same; Presence of the mother at home; Allergy in the mother; Streptococcal disease in the mother; Mother's profession; Mother's conditions of work; Conditions of hygiene of the same; Animals at home; Outside relationships of the child; Same of the parents; Travel and stays out of town; Allergy in the 1st, 2nd, 3rd and 4th child; Streptococcal disease in the 1st, 2nd, 3rd, and 4th child.

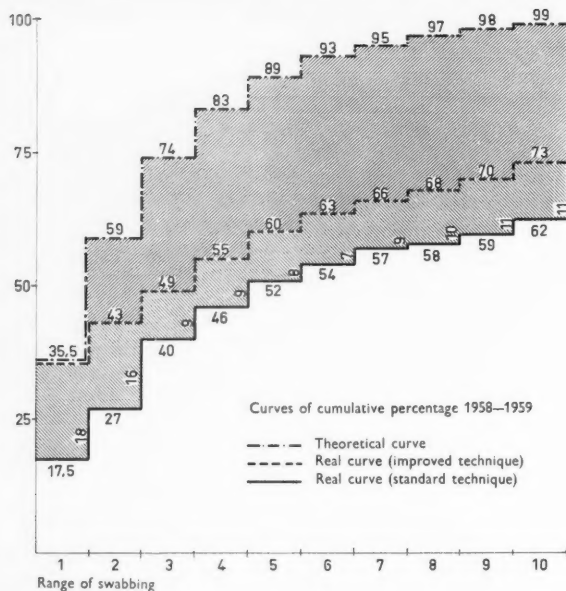


Fig. 8.

of the carriers, every subject being considered as a new carrier only the first time that a swab of his is found to be positive, whatever may be the result of subsequent swabbings.

Under these conditions we believe that the interpretation based on Fig. 6 (cf. Part I) remains valid, especially if it is considered that the real curve differs even more from the theoretical curve when it is corrected by taking the technique into account. It is seen in Fig. 5 (cf. Part I) that the number of new carriers is considerable, especially following the first two swabbings; this seems to us to be due to the fact that the technique being imperfect, two samplings are necessary in order to obtain the value of the average carrier percentage around which the variations will eventually be much smaller.

This conclusion seems to have been confirmed by the first results of another study now under way. Another group of children has been under study for 8 months in conditions rather similar to those of the above study but with a technical improvement which ensures more regular results. This new technique has in particular the effect of diminishing the number of false negatives, a fact which can be noted by studying the continuity of positive swabs in the carriers.

Figure 8 shows the results of the first 10 swabbings performed under these new conditions. It superimposes 3 curves: the theoretical curve of distribution according to chance, the curve observed with the standard technique, and that obtained with the improved technique. It will be seen that the latter while yielding a larger

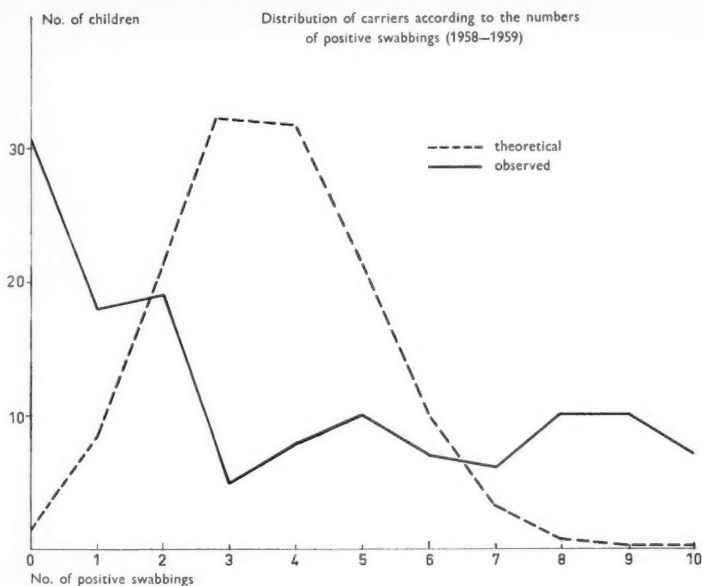


Fig. 9.

number of positive results as compared with the standard technique, does not produce a curve differing greatly from that given by the latter and that, although it differs notably during the first swabbings (a fact which confirms the argument set forth above), this divergence diminishes with accumulating results. While the lower hatched zone does represent a technical gain, there still remains a fundamental difference, represented by the upper hatched zone, between the theoretical distribution curve and the curve actually observed, and this difference demonstrates the presence of a carrier factor (or an anti-carrier factor).

It may, of course, be argued that an even finer technique would allow of further increasing the number of carriers. This is very likely, but it seems to us that,

in view of the very different shapes of the real and the theoretical curves and the fact that this difference is further enhanced by technical improvements (Fig. 8), such a purely technically obtained increase in the percentage of carriers would not succeed in altogether eliminating the class of subjects refractory to the carrier state.

Figure 9, constructed with the data of the improved technique shows clearly that the dispersion of carriers once, twice, three times ...  $n$  times is fundamentally different from a dispersion due to chance even when experimental error is greatly reduced.

We therefore feel justified in concluding that the interpretation of our findings demonstrates, in the course of a prolonged study, 4 types of behaviour with regard to

group A streptococci in a population of normal children: subjects who are never carriers, subjects who are occasional carriers, prolonged carriers of the same strain, prolonged carriers of frequently changing strains.

The limitations of the method seem to be of another kind and on this point our opinion is similar to that of the authors who have recently studied the subject (Saslow (23), Cornfeld & coll. (4)) These differences in behaviour of the subjects with regard to streptococci cannot now be investigated further simply by the detection of the organism in the throat, however sensitive the technique used. They probably correspond to constitutional differences in immunity for the study of which another method must be developed.

It is possible that the different techniques of organism detection may demonstrate different classes of carriers; it may, for example, be considered that the carriers discovered with the coarsest techniques are those who harbour organisms of greater vitality or in larger numbers. But this is a hypothesis which in any case would have to be confirmed by a parallel investigation. We believe for our part that one way of approaching the problem, as it arises now, would be to make a searching study of streptococcal immunity: it is this approach that has oriented our present investigations.

### Summary

In the course of a study pursued for 2 consecutive years in normal children of a suburban public school of the Paris area,

the following findings were noted: (1) when the technique used was sufficiently sensitive, the rate of healthy carriers was relatively high: 16.7 % on an average, with a range of 6.7 % to 30 %. (2) this percentage seemed to present variations during the course of the year which are perhaps due to a seasonal factor. (3) the cumulative percentage was such that at the end of two years more than one subject in two was found, to have been a carrier of hemolytic streptococci at least once. (4) there was no correlation between the rate of carriers and the development of clinical infections (septic sore throat, scarlet fever). (5) the subjects fell into 4 groups: refractory subjects, occasional carriers, prolonged carriers of the same strain, prolonged carriers of different strains. (6) a "prolonged carrier" factor was found to exist statistically, i.e. certain subjects were particularly receptive to the Group A hemolytic streptococcus, or conversely, certain subjects were refractory. (7) within the scope of our study this factor was found together with various other independent correlations. Of these, the correlations with the presence of tonsils and with bad conditions of hygiene (absence of running water in the child's home) were hardly surprising; that with a genetic factor (dark iris) was more unexpected, but has been established on equally as sound a basis.

### Erratum

On Fig. 6 and Fig. 8, the curves have been constructed with the total number of carriers instead of the cumulative percentages. This, however, makes no significant difference in the shape of the curves.



### Acknowledgements

This study would not have been possible without the help of Dr. F. Lazard. The statistical work has been performed under the direction of D. Schwartz, with the collaboration of M. Devaux. We are greatly indebted to Dr. Roman Pakula, National Laboratory of Hygiene, Warszawa, Poland, who has kindly performed the typing of several hundreds of our strains. Our thanks

also to Dr. René J. Dubos, the Rockefeller Institute for Medical Research, and to Pr. David D. Rutstein, Department of Preventive medicine, Harvard University Medical School, Boston, Mass., whose advice has been very useful.

Technical assistance: J. Lemaire; H. Gaugain; M. M. Sauzade; M. Goujon; F. Conté; C. Tassin; R. Erlichson; A. Tarnowska; A. Lemant; R. Julien; M. Rousselin; B. Coquelle; L. Beaupré and Z. Porgo.

### References

- BOURN, J. M., CAIPENTER, H. M. and MC COMB, E.: Streptococcus haemolyticus in the presence and absence of acute minor respiratory infection 1928-1931. *Amer J Hyg*, 17: 761, 1933.
- BUNN, W. H. and BENNET, H. N.: Community control of rheumatic fever. *JAMA*, 157: 986-989, 1955.
- Committee on Acute Respiratory Diseases. Problems in determining the bacterial flora of the pharynx. *Proc Soc Exp Biol Med*, 69: 45, 1948.
- CORNFIELD, D., WERNER, G., HUBBARD, J. P., WEAVER, R. and BELLOWS, M.: Streptococcal infection in a school population. *Ann Intern Med*, 49: 1305, 1958.
- MACDONALD, J., SIMMONS, R. T. and KEOGH, E. V.: Further observations on the incidence of hemolytic streptococci in the tonsils. *Med J Aust*, 1: 849, 1940.
- FELDMAN, H. A. and HARMON, W.: Comparative effectiveness of direct blood agar and modified Pike's broth for demonstrating  $\beta$  hemolytic streptococci of different serological groups. *J Lab Clin Med*, 40: 297, 1952.
- FRISCH, A. W.: Haemolytic streptococci from the throat of normal young adults. *J Infect Dis*, 62: 40, 1938.
- FRY, R. M.: An anaerobic method for identification of hemolytic streptococci. *J Path Bact*, 37: 337, 1933.
- HOLMES, M. C. and LERMIT, A.: Transport and enrichment media. *Monthly Bull Minist Health (Lond)*, 14: 97, 1955.
- HOLMES, M. C. and WILLIAMS, R. E. O.: The distribution of carriers of streptococcus pyogenes among 2413 healthy children. *J Hyg (Lond)*, 52: 165, 1954.
- KEOGH, E. V., MACDONALD, J., BATTLE, G. and SIMMONS, R. T.: The serological group and types of streptococci isolated from excised tonsils. *Brit Med J*, 2: 1036, 1939.
- LANCEFIELD, R. C.: The antigenic complex of haemolytic streptococcus. *J Exp Med*, 47: 91, 1928.
- PIKE, R. M.: An enrichment broth for isolating hemolytic streptococci from throat swabs. *Proc Soc Exp Biol Med*, 57: 186, 1944.
- PIKE, R. M.: The isolation of  $\beta$  hemolytic streptococci from throat swabs. *Amer J Hyg*, 41: 211, 1945.
- PIKE, R. M. and FASCHENA, G. T.: Frequency of streptococci in the throats of well children in Dallas. *Amer J Public Health*, 36: 611, 1946.
- POSKANZER, D. C. et al. Epidemiology of civilian streptococcal outbreaks before and after penicillin prophylaxis. *Amer J Public Health*, 46: 1513, 1956.
- QUINN, R. W., DENNY, F. W. and RILEY, H. D.: Natural occurrence of hemolytic streptococci in normal school children. *Amer J Public Health*, 47: 995, 1957.
- RAMMELKAMP, C. H.: In Streptococcal Infection. Edited by Macllyn McCarty—Columbia Univ. Press, New York 1954.
- RANTZ, L. A.: The haemolytic streptococci. Studies on the carrier state in the San Francisco area, with a note on methods of isolation and serological identification of these organisms. *J Infect Dis*, 69: 248, 1941.
- RILEY, H. D., QUINN, R. W. and DENNY, F. W.: Streptococcal carrier studies in children. *Amer J Dis Child*, 93: 64, 1957.
- RUBBO, S. D. and BENJAMIN, M.: Some observations on survival of pathogenic bacteria on cotton wool swabs. *Brit Med J*, 1: 982, 1951.
- RUSSEL, E. L.: Control of streptococcal throat infections in schools. A cooperative program followed in Orange County. *Calif. Med*, 85: 365, 1956.
- SASLAW, M. S. and STREITFELD, M.: (1953; 1953-54, 1954-55). Group A  $\beta$  hemolytic

- streptococci in relation to rheumatic fever. *Amer J Dis Child*, 92: 550, 1956.
24. WANNAMAKER, L. W.: American Heart Assoc. Pamphlet N.Y. 1956.
25. WANNAMAKER, L. W. *et al.* The single throat culture as an index of the bacterial flora of the respiratory tract. *Amer J Hyg*, 50: 168, 1949.
26. WERNER, G., CORNFELD, D., HUBBARD, J. P. and RAKE, G.: A study of streptococcal infection in a school population: laboratory methodology. *Ann Intern Med* 49: 1320, 1958.
27. WHEELER, S. M., JONES, T. D.: Factors in the control and spread of acute respiratory infection with reference to streptococcal illness and acute rheumatic fever. *Amer J Med Sci*, 58: 209, 1945.

Received Jan. 25, 1960

Accepted February 24, 1960

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## The Incidence of Head Injuries in Children

by JOHANNES C. MELCHIOR

In paediatric departments and, in particular, departments to which many patients are admitted with neurological and psychiatric disorders, information is frequently obtained in the case history that the patient has suffered from a head injury. The question arises: What is the value of this information and, just as important, how frequently does head injury occur in general in children? Only isolated numerical reports concerning the incidence of head injury in childhood are available and these are usually concerned with special categories of patients suffering from neurological and/or psychiatric symptoms.

Dyggve in a psychiatric material of children, obtained only scanty information concerning head injury. Otto from among 3588 children seen in psychiatric advisory clinics in Stockholm found that 20 per cent of all the patients had a history of head injury and that in  $\frac{1}{3}$  of the cases the injury had been associated with loss of consciousness. In this material boys were 3-4 times as common as girls. Melchior, in a follow-up investigation of children from a department of neurosurgery, found that 24 per cent of all the histories contained information about head injury.

This investigation was supported by a PHS research grant 3 B-9099 from the National Institute of Neurological Diseases and Blindness, U.S. Public Health Service.

It appears to be of interest, particularly in the evaluation of head injuries in neuro-psychiatric patient material, to determine the incidence of head injury among children without any such symptoms.

### Present Investigations

In order to obtain information concerning the incidence of head injury in children other than neurological and/or psychiatric patients, inquiries were made regarding head injury in all patients admitted to hospital over a period of eight months. The question: "Has the child ever had a head injury?" was put to the parents. If the reply was in the affirmative, inquiries were made concerning symptoms such as headache, vomiting, vertigo, loss of consciousness, whether a doctor was called, and treatment in a casualty department or admission to hospital if this occurred. During the eight month period, 1190 patients were admitted and in 56 cases the desired information was not obtained. In some cases this was due to the fact that there was simply no time to undertake routine questioning as the child concerned was desperately ill or for some other reason.

As already mentioned, it may be assumed that in a department which receives many children with neurological conditions (cerebral palsies, epilepsy, mental retardation etc.), information concerning head injury will occur more frequently in the case his-

TABLE 1. *Occurrence of history of head injury among children admitted with or without neuropsychiatric symptoms.*

	Girls				Boys			
Number admitted	471				663			
Neuro-psychiatric symptoms	- 377		+ 94		- 517		+ 146	
History of head injury	No	Yes	No	Yes	No	Yes	No	Yes
Number	337	40	65	29	448	69	108	38

tories and therefore this category of patients is separated in the present material. The presumption that head injury occurs more frequently among children with neurological or severe psychiatric conditions is due to several factors. It may be due to actual conditions, i.e. that head injury is genuinely more frequent than among children suffering from other conditions. It may also be due to the fact that parents of children suffering from neuropsychiatric conditions are more willing to answer questions regarding head injury positively than parents of children with other conditions. It frequently occurs that parents of the former category of children have thought a great deal about the condition and have remembered some injury or other which they consider may explain the condition of the patient. In addition, it is also more probable that the question has been asked previously on other admissions to hospital and they have had the opportunity of thinking about the problem. On the other hand, parents who come with a child suffering from pneumonia, otitis media or other conditions have probably not thought about head injuries and are probably less likely to consider whether such an injury had ever occurred.

In addition to subdivision into neuropsychiatric and non-neuro-psychiatric patients the patients are also divided according to sex in Table 1. It is frequently stated that boys are wilder than girls and that they are more frequently exposed to trauma than girls. Dyggve's figures from a department of child psychiatry

show that boys predominate in the ratio 2:1 over girls. Dyggve explains this by the fact that boys are more aggressive than girls and that this necessitates more detailed investigation.

Bohn, similarly reported that post traumatic lesions are more common in boys than girls and relates this to the fact that the behaviour of boys more frequently brings them into traumatic situations. In the present material it appears that the incidence in boys and girls is identical. In the group with neurological and psychiatric conditions the incidence for boys was 26 per cent against 31 per cent for girls and in the group with non-neurological conditions 13 per cent of the boys and 12 per cent of the girls had a history of head injury.

TABLE 2. *Comparison between percentage of head injuries and age.*

Age in years	Per cent of head injuries	
	Girls	Boys
> 6/12	17	18
> 1	18	18
> 2	19	22
> 3	20	22
> 4	20	20
> 5	21	27
> 6	23	20
> 7	25	29
> 8	26	20
> 9	29	27
> 10	31	27

TABLE 3. *Comparison between the severity of the head injury and the presence of neuropsychiatric symptoms.*

Head injury of:	Neuropsychiatric symptoms	
	absent	present
No importance	33 (30%)	27 (36%)
Possible importance: single symptoms etc.	44 (40%)	23 (28%)
Importance with unconsciousness, etc.	32 (30%)	27 (36%)

As the possibility of a history of head injury depends upon the age of the patient on admission, Table 2 shows the percentage of the children admitted over a certain age who had sustained head injuries. It will be observed from this that there is no particular difference between boys and girls. The boys possibly sustain their head injuries slightly earlier than the girls but, on the other hand, the incidence stabilizes about the age of 6-7 years while the incidence in girls rises more steadily throughout childhood.

The information collected was further subdivided with respect to the severity of the head injury into three groups. In Group 1 it must be supposed that the trauma has been without any significance. Only an affirmative answer was received and there were no symptoms at all. Among the patients with neuropsychiatric conditions, there were 27 in this group and among the non-neuropsychiatric 33. Group 2 comprizes such patients in whom genuine symptoms occurred in the form of vertigo, vomiting, headache and also cases where a doctor was called or the patient taken to a casualty department. In this group the figures are 23 among the neurological patients and 44 in the group of non-neurological

patients. In Group 3 there was definite loss of consciousness or the patient was admitted to hospital on account of the head injury and, by and large, the injury must be regarded as being of considerable severity. In this group, there were 27 patients from the neurological group and 32 from the non-neurological group. The figures in brackets in Table 3 indicate the percentage figure in relation to Groups 1, 2 and 3. It appears from the Table that in approximately one third of the cases in both categories the history of head injury can be ignored and it may be concluded that, in these cases, it has not played any significant role. In a further third the significance of the head injury is questionable and in the remaining third the trauma has probably been of such a nature that it might be responsible for symptoms later on. This corresponds to Otto's statement that approximately one third of the patients, about whom he had information, had been unconscious.

The fact that the distribution is so completely uniform in the groups with and without neuro-psychiatric symptoms may be regarded as indirect evidence that the value of the information obtained from the parents in both groups of patients is the same. This implies that the difference between the 12-13 per cent in the one group and approximately 28 per cent in the other group is a genuine difference and that, regardless of the group, approximately one third of the histories of head injury must be assigned to each of the three degrees of severity.

### Summary

In 1190 children consecutively admitted to hospital, inquiries were made con-

cerning head injury and information on the subject was obtained in 1134. In the entire material, 16 per cent replied in the affirmative and no difference in the incidence between boys and girls was observed. In patients admitted on account of neu-

rological or severe psychiatric conditions the incidence was significantly greater, viz 28 per cent as compared with 12 per cent in the group of patients without neurological or psychiatric conditions.

### References

- BOHN, S. S.: The reactions of patients to encephalography. *Bull Neurol Inst (New York)*, 6: 540, 1937.
- DYGGVE, H.: Nogle psykologiske synspunkter og

erfaringer vedrørende nervøse børn og deres forældre. *Ugeskr Læg*, 119: 105, 1957.

MELCHIOR, J. C.: To be published.

OTTO, ULF: The postconcussion syndrome in children. *Acta Paedopsychiat*, 27: 5, 1960.

Received April 13, 1960

Accepted May 12, 1960

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## Umbilical Vein Pressure in Congestive Heart Failure in the Newborn Infant\*

by PAUL M. TAYLOR, JEROME H. WOLFSON, NANCY H. BRIGHT  
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The diagnosis of congestive heart failure in the newborn infant is usually based on clinical findings. Respiratory distress and hepatomegaly are the earliest and most constant signs. Cyanosis, splenomegaly, peripheral edema and rales are noted later in most cases or may not develop at all. Cardiac enlargement is often detected clinically and confirmed by roentgenogram of the chest.

The clinical diagnosis of heart failure may be difficult to make with certainty in some infants with hemolytic disease, the respiratory distress syndrome, septicemia or pneumonia—to mention a few common complications of the newborn period. These conditions may all, of themselves, give rise to combinations of clinical findings that strongly suggest cardiac failure. Heart failure may, on the other hand, complicate the course of these entities. We have found determination of umbilical vein pressure helpful in evaluating infants in whom the diagnosis of cardiac failure is considered. This report presents our experience with such infants.

\* Supported by the Twenty-Five Club, a Woman's Auxiliary of the Elizabeth Steel Magee Hospital.

### Patient Material and Method

An attempt was made to measure umbilical vein pressure on all infants delivered at the Elizabeth Steel Magee Hospital over a period of 18 months on whom the diagnosis of cardiac failure was considered. It was not possible to do so in a few cases. Ten infants were studied. Their ages at time of determination of venous pressure ranged from 22 to 75 hrs. Data on infants with severe hemolytic disease are not included in this report.

Umbilical vein pressure was measured in mm of normal saline with the mid-axillary line used as zero position for the manometer. The stump of the umbilical cord was incised and the umbilical vein entered with a polyethylene or polyvinyl catheter which was then advanced from 4 to 7 cm. Readings were accepted when the infants seemed maximally relaxed, and when the fluctuations normally seen with respiration were noted. Repeated measurements were made until consecutive readings agreed within a few mm.

### Results

Pertinent information on the infants presented is listed in Table 1. Their umbilical vein pressures are compared with umbilical vein pressures of healthy infants (2) in Fig. 1. Seven of the 10 infants had

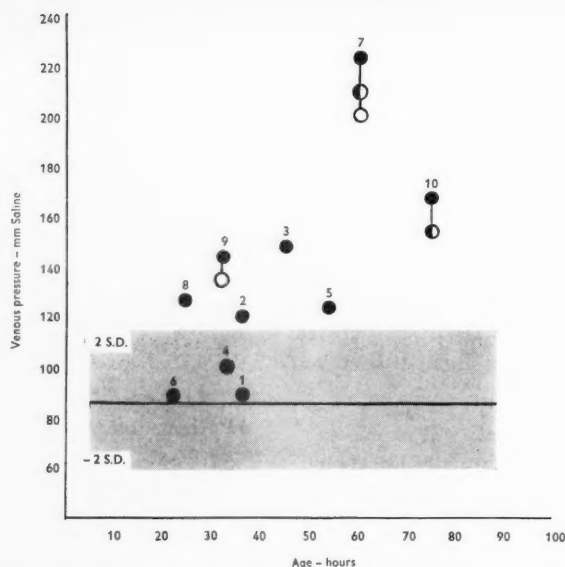


Fig. 1. Umbilical vein pressures of infants on whom the diagnosis of cardiac failure was considered. The heavy horizontal line indicates the mean venous pressure (92 mm saline) of 31 healthy infants 5-89 hours old. The shaded area represents  $\pm 2$  S.D. from the mean venous pressure (2). The numeral above each point refers to the case number noted in Table 1. Half-closed circles and open circles indicate values obtained after removal of a total of 10 ml and 20 ml of blood, respectively.

venous pressures greater than 118 mm (2 standard deviations above the mean venous pressure for healthy infants). The incised umbilical vein bled briskly in these infants with elevated venous pressure. Six of these 7 infants died, while 2 of the 3 infants with venous pressure within the normal range survived.

Withdrawal of 10 to 20 ml of blood from the umbilical vein of 3 of the infants in heart failure resulted in an immediate drop in venous pressure (Fig. 1). Following venesection, temporary reduction of dyspnoea and of liver size was noted in Case 7, but clinical improvement did not occur in Cases 9 and 10.

Umbilical vein catheterization did not produce any discernible adverse effects on the infants in this series.

## Discussion

Elevated umbilical vein pressure indicates failure of the right ventricle with these 3 possible exceptions: 1. during the first hour or so of life following delayed clamping of the umbilical cord (2); 2. when associated with marked abdominal distention which may, of itself, increase umbilical vein pressure; and 3. with obstruction of the inferior vena cava above the site of entrance of the hepatic vein and ductus venosus.

The clinical impression of congestive heart failure was confirmed by the finding of elevated venous pressure in 7 of the 8 infants presenting with the triad of dyspnoea, tachypnoea and hepatomegaly. The one subject with these findings and a

TABLE 1. *Information on Infants with Findings Suggestive of Congestive Heart Failure.*

Case number	Weight in g	Age onset of distress in hours	Pertinent findings								Age at venous press. in hours	Age at death in hours	Diagnosis Autopsy findings Remarks
			Tachypnea	Dyspnea	Hepatomegaly	Splenomegaly	Rales	Edema	X-ray	Venous pressure in mm saline			
1	4230	1½	+	+	-	-	-	-	C <sup>a</sup>	95	36		Postmaturity, Recovered.
2	5870	At birth	+	+	+	-	-	-	X <sup>b</sup>	120	36		Respiratory distress syndrome, Recovered.
3	3000	40	+	+	+	-	-	-	C	155	45	50	<i>Aerobacter aerogenes</i> septicemia.
4	3390	28	+	+	+	-	+	+	C	100	33		Acute pulmonary edema, Mother diabetic, Recovered.
5	3055	24	+	+	+	+	-	+	C	125	54	55	Hemorrhagic pneumonia, Vascular congestion, Mother diabetic.
6	2500	18	+	+	-	-	+	-	ND <sup>c</sup>	94	22	24	Atelectasis and pulmonary hyaline membrane.
7	3775	48	+	+	+	+	+	-	C	225	60	70	Aortic and mitral atresia, absent left ventricle, hypoplastic left auricle and ascending aorta.
8	3550	At birth	+	+	+	-	-	-	C	135	24	37	Cor triloculare, Bonnevie-Ullrich syndrome.
9	3855	8	+	+	+	-	-	-	C	145	32	46	Bronchopneumonia.
10	3775	72	+	+	+	-	-	-	C	175	75	80	Cor triloculare, truncus arteriosus, mitral atresia.

<sup>a</sup> Cardiomegaly<sup>b</sup> Normal cardiac shadow<sup>c</sup> X-ray not taken.

normal venous pressure (Case 4), the infant of a diabetic mother, had a moderately enlarged liver from birth. Two infants in this series had labored and rapid respiration and other findings suggestive of heart failure, but normal-sized livers. Both had venous pressures within the normal range.

Walther (3) has reported the case of an infant of a diabetic mother who developed the clinical picture of the neonatal respiratory distress syndrome between the ages of 2 and 5 hrs, and who had an elevated umbilical vein pressure at 5 hrs. of age. With-

drawal of 60 ml of blood lowered the umbilical vein pressure to within the normal range and promptly cured the infant of his respiratory difficulty. Case 4, the infant of a diabetic mother, had onset of acute pulmonary edema at 28 hrs. of age; his umbilical vein pressure was normal at that time. He improved rapidly after withdrawal of 20 ml of blood, which did not affect his venous pressure, and digitalization. This may have been a case of acute left ventricular failure, which is seldom diagnosed with certainty in the newborn infant. These experiences suggest that venesection merits a therapeutic trial in severely distressed infants of

diabetic mothers, no matter what the infant's venous pressure is found to be.

Bonham Carter, Bound & Smellie (1) measured umbilical vein pressures during the first hours of life in a series of infants with the neonatal respiratory syndrome. They found that over half of the infants that survived had elevated venous pressures, while all of the infants who died had venous pressures within the normal range. The explanation of the seemingly beneficial effect of elevated venous pressure in these infants is obscure.

Two full term infants with neonatal respiratory distress are included in the present report. Case 2, with a slightly elevated umbilical vein pressure at the age of 36 hrs, survived, while Case 6, whose venous pressure was normal at 22 hrs. of age, died.

Cardiac failure in the newborn infant was found to be associated with a variety of clinical conditions in this study. The incidence of congenital heart disease as a cause of heart failure in newborn infants

is probably higher than the 3/7 noted in this small series. It is important to appreciate that congestive failure may complicate both septicemia and pneumonia in infants. Early diagnosis and treatment of this complication in such cases may be life saving.

When the clinical impression of cardiac failure is confirmed by the finding of elevated umbilical vein pressure, we slowly withdraw sufficient blood (up to 30 ml in mature infants) to effect a definite lowering of venous pressure, and then inject the initial dose of Cedilanid® through the catheter. The ill infant is thus saved the stress of injection and venepuncture.

### Summary

Information is presented on 10 infants on whom the diagnosis of cardiac failure was suspected clinically. Measurement of umbilical vein pressure was made in each case, and proved to be of diagnostic value.

### References

1. BONHAM CARTER, R. E., BOUND, J. P. and SMELLIE, JEAN M.: Mean venous pressures in the first hours of life. *Lancet*, 271: 1320, 1956.
2. TAYLOR, P. M., EGAN, T. J., BIRCHARD, EDNA L., BRIGHT, NANCY H. and WOLFSON, J. H.: Venous hypertension in the

newborn infant associated with delayed clamping of the umbilical cord. In preparation.

3. WALTHER, T.: Is acute heart failure the cause of the neonatal-syndrome in children born of diabetic mothers? A preliminary report. *Ann Paediat (Basel)*, 189: 26, 1957.

Received May 5, 1960

Accepted June 15, 1960

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## Diarrhoea Caused by Deficiency of Sugar Splitting Enzymes. I

by H. A. WEIJERS, J. H. VAN DE KAMER, W. K. DICKE and J. IJSSELING

### Introduction

In daily practice we are often confronted with diarrhoeas whose cause cannot be found, causal therapy therefore being impossible in these cases. Recently we had to deal with these types of diarrhoea in three children admitted to our clinic.

*Patient X*, a girl, was born on July 15th, 1957 as the first child of healthy parents, delivery was normal, at term. Birth weight 3500 g. After one week of breastfeeding, Lactogen feeding was given, on which the child grew well with normal defaecation. When 5 months old she suddenly fell ill with high fever and diarrhoea. One day later the fever abated, but the diarrhoea persisted in spite of various therapeutic measures (antibiotics, Arobon, buttermilk, protein milk, wheat-free diet, etc.). Her increase in weight, which had been normal for 5 months, was now arrested.

At admission—at the age of 14 months—the child was dystrophic with a weight of 9900 g and a height of 80 cm; she produced a profuse, thin, foaming and voluminous diarrhoea that contained much fine mucus. She had a rather bad mood, was petulant and somewhat negativistic. Her muscular development was normal, and physical examination revealed only a somewhat flaccid and swollen abdomen, and rather lively intestinal noises were heard on auscultation.

The following conditions were eliminated as causes of the diarrhoea; enteric infections (amoebic and bacillary dysentery, typhoid

and paratyphoid, coli Bray and other pathogenic intestinal flora), worms and giardiasis, coeliac disease, anatomical abnormalities of the gastro-intestinal tract, terminal ileitis, latent iron deficiency, vitamin deficiencies and pancreatic fibrosis, while congenital alkalosis, milk allergy and ganglioneuroma, were discarded as causative factors.

*Patient Y*, boy, was born one month prematurely on October 6th, 1957. Delivery was normal, birth weight was 2300 g. Up to six months he was breastfed with good growth and no diarrhoea. Then persistent diarrhoea (rather thin faeces, yellow, foaming, 2 or 3 times per day).

When, two years old, the boy was admitted to hospital, he was a moderately well, lively, palish child with a somewhat swollen abdomen and slight general hypertonia. During periods of diarrhoea he was unruly and whimpering. On feeding with a protein-milk/water/glucose mixture the diarrhoea disappeared and the boy became cheerful again. Deviations from this diet always caused diarrhoea. Here also all known causes of diarrhoea were ruled out.

His weight on admission was 9800 g (at the age of six months it had been 6500 g) and his height was 77 cm. He was the second child of healthy parents. The first child had died at the age of 15 months from meningitis.

*Patient Z*, a boy, was born on August 12th, 1958. Delivery at term was normal and birth weight was 4300 g. He was breastfed for a few days only and diarrhoea commenced

after the first few weeks of life, both on diets of half milk/half water/buttermilk mixtures and on acid/whole milk mixtures; he produced normal stools only on a protein-milk/water/glucose mixture. The diarrhoea was foamy and water-thin and contained some fine mucus.

The patient was admitted at the age of one year, because of the persistent tendency to develop diarrhoea as soon as he was given a diet other than the protein-milk/water/glucose mixture. All known causes of diarrhoea were excluded.

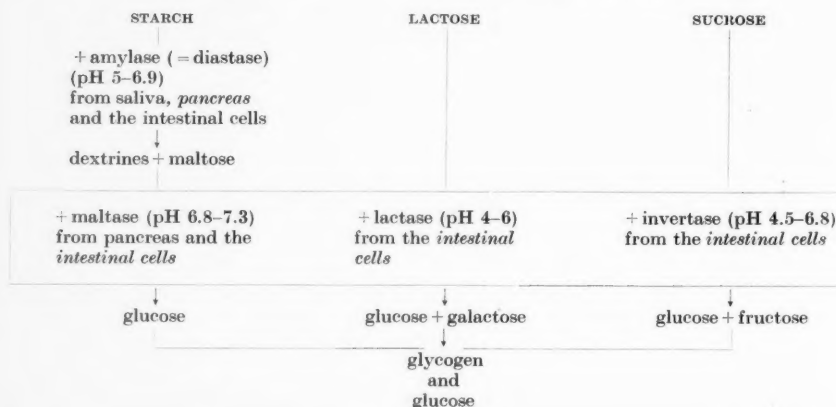
On admission he was a pale baby weighing 8700 g and 75 cm in height. Examination did not reveal any abnormal physical signs, apart from a somewhat swollen abdomen. The patient was the fourth child of healthy parents. The eldest child also suffered from diarrhoea.

As the diarrhoea observed in the three patients was thin, foaming and bulky, and contained very finely distributed mucus, which is typical for a diarrhoea originating in the small intestine (Goiffon), we had to give serious thought to an absorption disturbance as causative factor. Especially in view of the foaming and the low pH of the faeces we thought of fermentation as a

result of a disturbance in carbohydrate absorption. The nutritional components to be suspected in this connection, are starch, lactose and saccharose, as these substances as such cannot pass the intestinal wall,—in contrast to the monosaccharides glucose, galactose and fructose—but are first split in the intestine into monosaccharides: glucose, glucose + galactose and glucose + fructose, respectively (see Scheme I), followed by absorption. However, if the carbohydrate-splitting enzymes amylase (= diastase), maltase, lactase and invertase (= saccharase) are absent or insufficiently active, starch (dextrins, maltose), lactose and saccharose may reach the ileocaecal region and there be attacked by the intestinal bacteria, to cause a vigorous fermentation with formation of lower organic acids, with harmful results.

That one has to reckon with these considerations became clear when Holzel *et al.* published recently about 2 children suffering from chronic diarrhoea caused by lactase deficiency. Some cases of starch

SCHEME I. *Splitting of carbohydrates for absorption*





intolerance classified by Andersen as coeliac disease are probably also caused by amylase and/or maltase deficiency.

### Investigations

Carbohydrate tolerance tests and faecal analysis can be used to trace possible deficiencies in carbohydrate-splitting enzymes as causes of diarrhoea.

#### a. Carbohydrate tolerance tests

In a loading test the child—in the fasting condition—is given 2 g of the carbohydrate under test per kg body weight—maximally 50 g—in a 10 % aqueous solution. Then we determined whether the reducing power of the blood increases or not.

Subsequently the enzyme preparation concerned is added to the sugar, the child is given this mixture with cold water and the curve is determined a second time.

The starch loading test is already described by *Althausen*, analogous to the casein loading test.

To test the lactase activity in the intestine, the lactose curve was first applied by *Holzel* and co-workers.

If the curve only rises with the addition of an enzyme preparation serious thought should be given to an enzyme deficiency.

When interpreting curves, one should realize that their course depends not only

on the rate of supply of monosaccharides to the blood, but also on the rate of removal of these substances from the blood. Supply and removal are, in turn, resultants of various factors (Scheme II).

It was our experience in several children that a flat loading curve was caused by a marked carbohydrate hunger of the liver. This phenomenon could be corrected by giving the patient an extra amount of glucose during 5 days.

Some cautiousness is therefore warranted in the interpretation of the various curves. Usually, however, they can be evaluated as follows (Schemes III and IV).

The situation is normal if, after loading with 2 g lactose, maltose or saccharose per kg body weight, an increase of the reducing power of the blood serum is found of approximately 50 mg %, expressed as glucose. This means that both the enzyme activity and the absorption are good.

If, however, the curve rises less than 20 mg %, we should expect a too low lactase, maltase or invertase activity, or a disturbance in the absorption. To find out which is responsible, the curves are repeated but now with addition of the enzyme concerned. If the curve rises after this addition, the assumption of a too low enzyme activity in the intestine, is justified. If, however, the curve remains flat in this case also, the loading should be repeated once more, namely with the monosaccharides which form the elements of lactose, maltose and saccharose;

SCHEME II. *Factors influencing the course of a carbohydrate loading curve*

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#### SUPPLY

- a. dosage
- b. speed of absorption
  - 1. speed of gastric emptying
  - 2. fermentative splitting in the intestine
  - 3. absorption by the cells of the intestinal wall and transport through the portal vein
- c. glycogenolysis

#### REMOVAL

- a. glycogen synthesis
  - b. direct combustion in the tissues
  - c. threshold value of the kidney
-

## SCHEME III.

*Lactose tolerance test*  
(L.T.T.)

an increasing curve:  
NORMAL LACTASE ACTIVITY  
AND NORMAL ABSORPTION

a flat curve:

loading with glucose + galactose

an increasing curve:  
LACTASE DEFICIENCY  
proof: loading with lactose +  
lactase: increase

a flat curve:  
DISTURBED ABSORPTION

*Sucrose tolerance test*  
(Su.T.T.)

an increasing curve:  
NORMAL INVERTASE ACTIVITY  
AND NORMAL ABSORPTION

a flat curve:

loading with glucose + fructose

an increasing curve:  
INVERTASE DEFICIENCY  
proof: loading with sucrose +  
invertase: increase

a flat curve:  
DISTURBED ABSORPTION

## SCHEME IV.

*Starch tolerance test*  
(St.T.T.)

an increasing curve:  
NORMAL AMYLASE- AND MALTASE-  
ACTIVITY AND NORMAL ABSORPTION

a flat curve:

A. DISTURBED ABSORPTION

proof: loading with glucose: no increase or/and B. MAL-  
TASE and/or AMYLASE DEFICIENCY

followed by a

*Maltose tolerance test*  
(M.T.T.)

an increasing curve:  
AMYLASE DEFICIENCY  
proof: loading with starch + amylase:  
increase

a flat curve:

A. MALTASE DEFICIENCY

proof: loading with starch + maltase: increase

B. MALTASE AND AMYLASE DEFICIENCY

proof: 1. loading with starch + maltase: no increase  
2. loading with starch + maltase + amylase:  
increase

in other words, with a mixture of equal amounts of glucose and galactose, and glucose, and glucose and fructose, respectively. In this case a flat curve points to a disturbance in the absorption mechanism.

As the breakdown of starch to the monosaccharide glucose takes place in two stages, for which two enzymes are required, i.e. amylase and maltase, a loading with starch—2 g per kg bodyweight, with a maximum of 50 g—which results in an increase of the reducing power of the blood serum of about 50 mg %, expressed as glucose, points to a good amylase and maltase activity, as well as to a normal absorption.

If the curve of the starch tolerance test (St.T.T.) does not rise, however, we should again differentiate which of the three factors mentioned just now is responsible.

The absorption is to be judged by means of a simple glucose load; a flat curve points to a disturbance in the absorption.

If the glucose tolerance test (G.T.T.) shows a normal rise, however, the loading should be done with maltose, as a flat maltose tolerance test (M.T.T.) in this case indicates a maltase deficiency, while in the case of a normally rising M.T.T. the maltase activity is good. A deficient maltase activity can even be demonstrated in more detail by repeating the M.T.T. once more, but now under the addition of maltase. Now the curve should show a normal rise. A maltase depletion may cause a flat St.T.T. curve, which can be in-

vestigated by loading with starch under addition of maltase. The curve should then rise.

Besides a maltase deficiency, there may also be an amylase depletion. In this case loading with starch to which maltase has been added will not cause a rise, while addition of maltase and amylase will do so.

If both G.T.T. and M.T.T. show a normal rise, the flat St.T.T. can only be caused by an amylase deficiency. This is to be proved by once again loading with starch, but in this case only with addition of amylase; the curve showed then rise.

#### b. Examination of the faeces

Based on a diet that only contains glucose as a carbohydrate—and after previous demonstration that the glucose absorption is good—the harmful activity of the carbohydrates starch, maltose, lactose or saccharose can be tested clinically, using the occurrence of diarrhoea as criterion, by replacing the glucose by an equivalent amount of one of these carbohydrates.

Moreover, chemical examination of the faeces may afford an understanding of the severity and nature of the disturbance (chemical methods, see Addendum).

Bacteriological examination can be carried out by using a direct quantitative typing of the more significant components of the faecal microflora as diagnostic criterion. For this purpose the viable numbers of the fol-

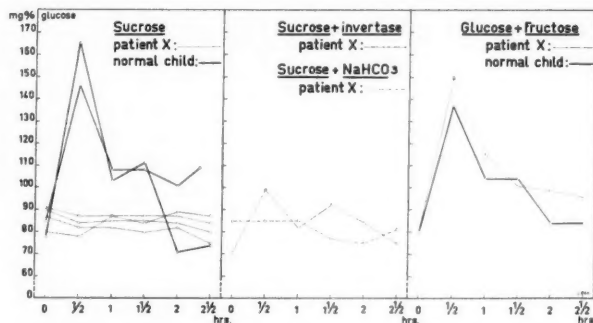


Fig. 1. Sucrose tolerance test in invertase deficient patient X.

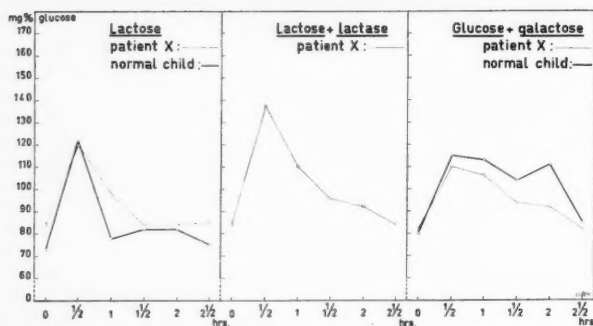


Fig. 2. Lactose tolerance test in non lactase deficient patient X.

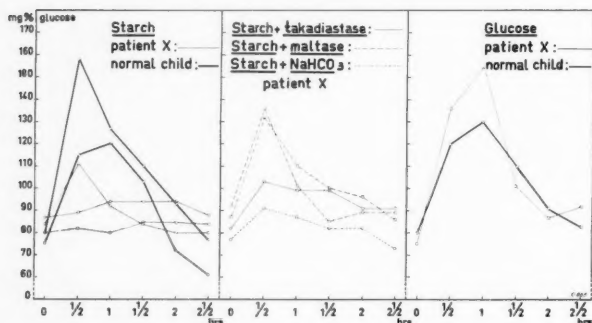


Fig. 3. Starch tolerance test in non amylase deficient patient X.

lowing organisms were determined in fresh stool samples both during episodes of diarrhoea and while recovering:

- (i) three broad general classes of bacteria;
- (ii) four typical groups of faecal bacteria;
- (iii) yeasts

### Results of the examinations

#### a. Carbohydrate tolerance curves

*Patient X.* After *saccharose* loading there was a flat curve, which points to an *invertase deficiency*, an absorption disturbance being excluded because loading with glucose + fructose caused a normal rise. Some rise was indeed observed if the load-

ing was done with *saccharose* to which *invertase* had been added (Fig. 1).

After loading with *lactose*, a normally rising curve was determined, which means that a *lactase deficiency* was to be ruled out (Fig. 2).

A flat curve was found after loading with *starch* (Fig. 3), as also after loading with *maltose* (Fig. 4), at any rate in January. In September, however, some rise was observed. This was not caused by a disturbed absorption, as the glucose tolerance curve showed a normal rise (Fig. 3). Neither was it possible to explain the flat course of the starch loading curve by

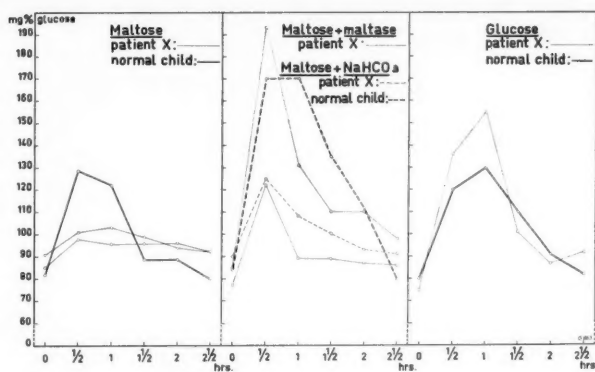


Fig. 4. Maltose tolerance test in maltase deficient patient X.

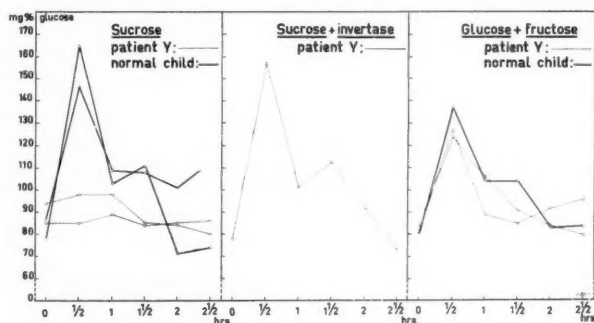


Fig. 5. Sucrose tolerance test in invertase deficient patient Y.

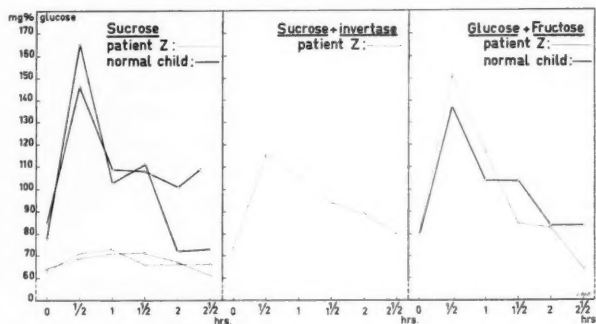


Fig. 6. Sucrose tolerance test in invertase deficient patient Z.

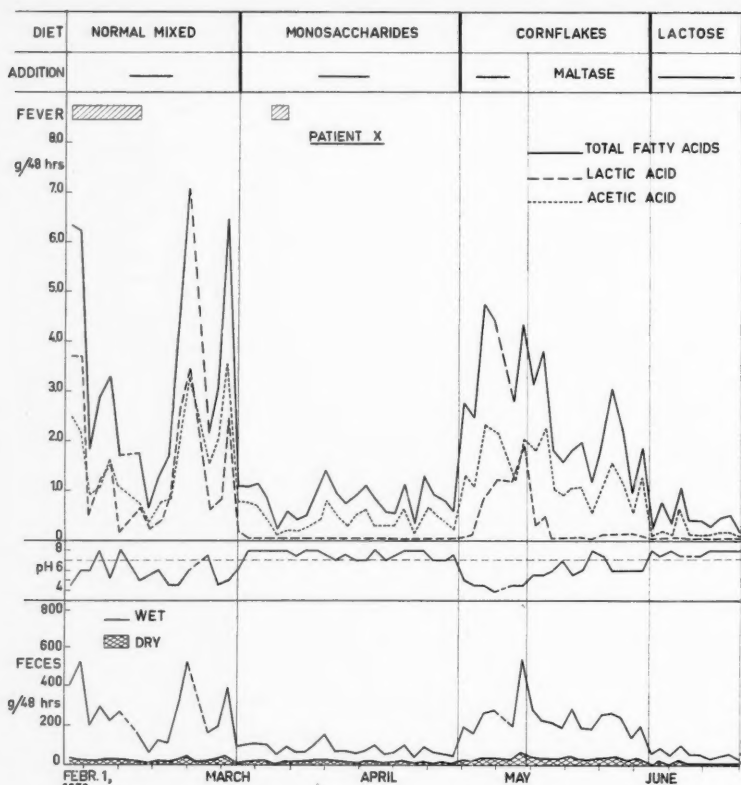


Fig. 7. Faeces examination in patient X, suffering from maltase and invertase deficiency, on a basic diet supplemented successively with different carbohydrates without and with enzymes added from February till June.

assuming an amylase deficiency, as a normal quantity of amylase was determined by means of duodenal sounding. Moreover, the starch tolerance curve was also flat with addition of takadiastase (Fig. 3). The explanation should therefore be sought for in a *maltase deficiency*. Loading with maltose, to which maltase had been added, indeed resulted in a normally rising curve, while a normal rise was also obtained by loading with starch to which maltase had been added (Fig. 3).

*Patients Y and Z.* A flat saccharose to-

lerance curve was also determined in these children; the curve did rise if invertase was added during the loading (Figs. 5 and 6). As the rise of the two glucose-fructose curves was normal, the conclusion was: *invertase deficiency*.

In none of the two patients could a lactase, maltase or amylase deficiency be demonstrated.

#### b. Analysis of the faeces

In patient X, the various diet periods showed the following trend as regards the

Fig. 8.



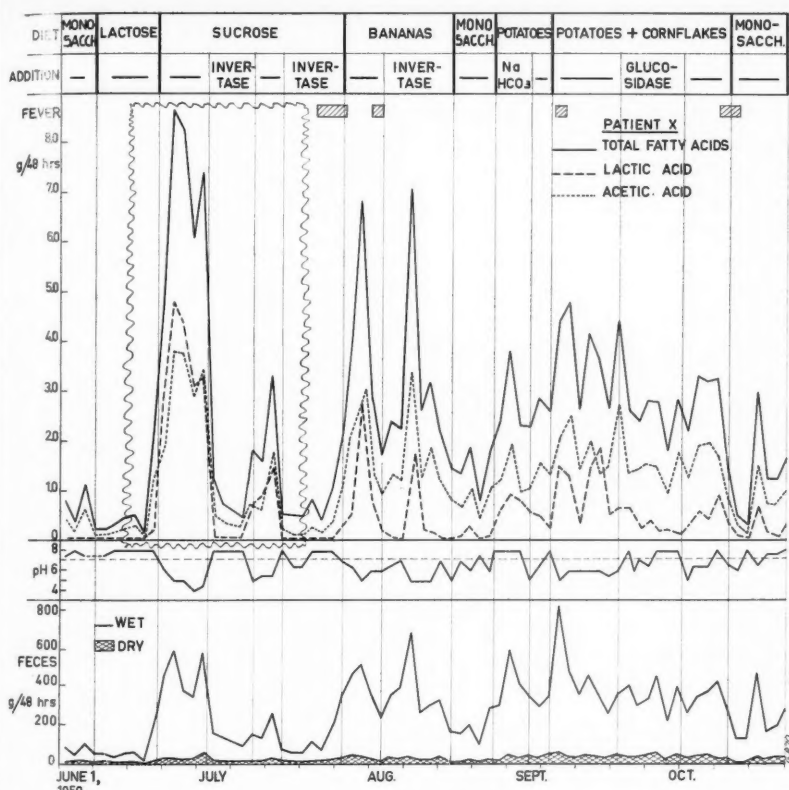


Fig. 8. Continuation of Fig. 7, from June till October; the encircled part is given more detailed in Fig. 10.

quantities of faeces and its composition (Figs. 7 and 8).

As shown in the above figure, the child suffered seriously from diarrhoea when being on a normal mixed diet. She produced up to 500 g faeces per two days, while she also excreted a considerable proportion of lower organic acids, mainly acetic acid and lactic acid, increasing up to 7 g per 48 hours; these acids are a criterion for the activity of the fermentation flora in the intestine, which, in this case, was

abnormally great. It is therefore no cause for wonder that the pH of the faeces was low, even as low as pH 4.5. The fat excretion per 48 hours was only slightly above normal.

The patient was subsequently given a diet that contained glucose as the only carbohydrate. This diet was composed as follows:

- 7.30 a.m.: 150 g curds + 25 g Nutroma + 35 g glucose
- 9.30 a.m.: the press juice of one orange

11.30 a.m.: 100 g green vegetables +  
30 g lean meat + one spoon-  
ful of gravy

2.30 p.m.: like 7.30 a.m.

5.30 p.m.: like 7.30 a.m.

This diet led surprisingly rapidly to a markedly favourable effect. The volume of the stools decreased to less than 100 g per 48 hours, the acid excretion fell from 7 g to 1 g and the pH rose to 8. The fat excretion, too, decreased to entirely normal values.

The improvement was also striking in clinical respect. Her negativistic, slightly irritated adjustment to her surroundings disappeared and she became a cheerful, active little girl.

The glucose-containing diet was continued for  $1\frac{1}{2}$  months, during which period the girl remained in an excellent condition without producing diarrhoea.

Then the diet was changed only in this respect that a part of the glucose in the diet was replaced by a calorically equivalent quantity of starch in the form of ground cornflakes, a product consisting largely of starch and which can easily be used in the diet instead of glucose, by mixing it with the curds. The patient reacted promptly to this change with a considerable rise of the amount of lower organic acids and a corresponding fall of the faecal pH, shortly afterwards followed by diarrhoea.

In view of the result of the duodenal intubation and the flatness of the curve after loading with starch to which taka-diastase had been added, the expectation was justified that the diarrhoea was not caused by an amylase deficiency but rather by a shortage of maltase and a maltase-containing preparation was there-

fore added to the diet, in a dosage of 18 g three times per day, given after one-third of the meal had been eaten. A favourable influence was observed, the diarrhoea decreased, while the excretion of lower organic acids also became somewhat less, especially when the quantity of maltase preparation was raised up to 27 g three times daily.

Although an improvement was perceptible, it was not until reverting to the glucose diet as used in the previous period that it became evident that this improvement had been only partial, as the glucose diet was directly followed by a further favourable reaction, to such a degree that the diarrhoea disappeared entirely, as also the increased acid excretion and the faecal pH rose. In our opinion, the cause of the moderate change for the better after administration of maltase, finds an explanation in the fact that maltase is very acid-sensitive, resulting in a considerable loss of activity during the passage through the stomach.

When, subsequently,  $3 \times 12$  g of the glucose of the diet was replaced by  $3 \times 12$  g lactose, this had no harmful sequelae. This confirmed the expectation that there was no lactase deficiency—an expectation formed already on the basis of the lactose loading curve.

Finally,  $3 \times 12$  g glucose of the glucose-diet was replaced by an equal amount of saccharose. In view of the saccharose loading curve, diarrhoea was again to be expected; it did in fact occur, immediately and very violently, accompanied by a considerable excretion of lower organic acids, the pH of the faeces falling as low as 4.

The favourable reaction after addition

of 4-250 mg of an invertase preparation occurred as promptly as the unfavourable effect of saccharose without invertase.

The experiment was repeated once more, in general with the same result. It was this time shown that even half the amount of invertase had a favourable effect.

Now overripe bananas were added to the diet (bananas contain saccharose and little or no starch, especially when overripe) to which the patient reacted immediately and violently with diarrhoea and markedly increased acid excretion.

Addition of invertase had the expected favourable effect. In the beginning the diarrhoea returned, however, but disappeared again although no special measures were taken. Some days later it transpired that the unexpected aggravation of the diarrhoea and of the acid excretion had been caused by the eating of sweets containing several grammes of sucrose, which the patient had been given by her grandmother in spite of our prohibition.

As a deficient enzyme activity might not only be caused by an enzyme shortage but also by too low a pH in the duodenum, starch was subsequently given—as potatoes—with addition of  $3 \times 750$  mg NaHCO per day. This addition had, however, no favourable influence.

We also studied whether the enzyme glucamylase exerted a favourable influence on the digestion of starch, as this enzyme is less susceptible to the influence of gastric acid than maltose. However, the breakdown of starch by glucamylase differs from that by amylase + maltase, as glucamylase at once splits off glucose without the formation of maltose as an

intermediate product. It is therefore capable of compensating a maltase shortage.

As, however, the pancreas activity of the patient was normal, a competition between the amylase and glucamylase activities was to be expected. In other words, there was a fair chance that, in spite of the presence of glucamylase, maltose would still be formed as glucamylase is able to break down starch directly to glucose, but leaves maltose intact.

Glucamylase had indeed no favourable effect; the diarrhoea and the high acid excretion persisted; on the other hand, the lactic acid excretion decreased under the influence of glucamylase.

The original glucose diet was finally given again, following which the diarrhoea disappeared.

As we therefore did not succeed in remedying the deficient maltase activity by some preparation or other, the child was discharged on the glucose diet. This may be supplemented with saccharose, provided invertase is also given. The child (Patient X) is in very good condition on this diet and no longer suffers from diarrhoea.

Patients Y and Z (Fig. 9) showed the same rapid intense reaction to saccharose as patient X. They also showed a prompt recovery after addition of invertase to the diet.

On *differentiation of the lower organic acids* in the diarrhoea periods (see Fig. 10) it appears that the faecal fatty acids consist of more than 90 % of lactic acid and acetic acid. A particularly remarkable feature is the high excretion of lactic acid, as this acid was almost entirely absent in all three patients in periods

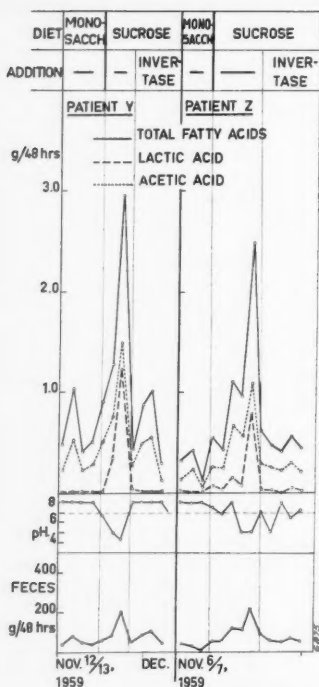


Fig. 9. Faeces examination in patients Y and Z, suffering from invertase deficiency; on a basic diet supplemented with monosaccharides and sucrose, resp.

without diarrhoea. The acetic acid excretion runs parallel to that of lactic acid, but is not entirely absent in periods free of diarrhoea.

As regards the acids excreted to a much less degree than lactic acid and acetic acid, the excretion of formic acid and perhaps also of isobutyric acid runs parallel to the lactic acid and acetic acid excretion. The behaviour of propionic acid and butyric acid is quite different, however, and there is no excretion at all of valeric acid and isovaleric acid in diarrhoea periods in which lactic acid and acetic acid are excreted in such abnormally

great amounts. Finally, neither in normal periods nor in diarrhoea periods was it possible to demonstrate caproic acid ( $C_6$ ).

The first change observed during the onset of a diarrhoea period is an increase in the acetic acid excretion; thus the pH in the intestinal lumen is lowered and it seems acceptable to suppose that thus the growth of a lactic acid-forming bacterium is made possible, but this growth entirely disappears again as soon as the pH begins to rise due to a decrease of the fermentation, viz. when the disaccharides are split and therefore absorbed, less carbohydrates being offered to the intestinal flora.

In view of the data collected so far it seems probable that especially the lactic acid excretion is a criterion for abnormal fermentation caused by disturbed carbohydrate absorption. It is therefore very fortunate that this acid can separately be determined in a relatively simple and rapid way.

The results of the bacteriological examination are given in Table 1; it may be stated here that in no case either *Salmonellae* were observed or numbers of *Staph. aureus* exceeding the order  $10^2$  per gramme.

Increases in total counts and count of faecal streptococci and *Bifidobacterium bifidum* and decreases in counts of aerobic proteolytic bacteriae, *Enterobacteriaceae* and sulphite-reducing clostridia, were generally noticed when the patients showed diarrhoea. However, these bacteriological changes, with the exception of the increase in numbers of faecal streptococci during diarrhoeal episodes, never reached the same order of magnitude and therefore of diagnostic reliability, as the decrease in pH or increase in lactic

Fig.

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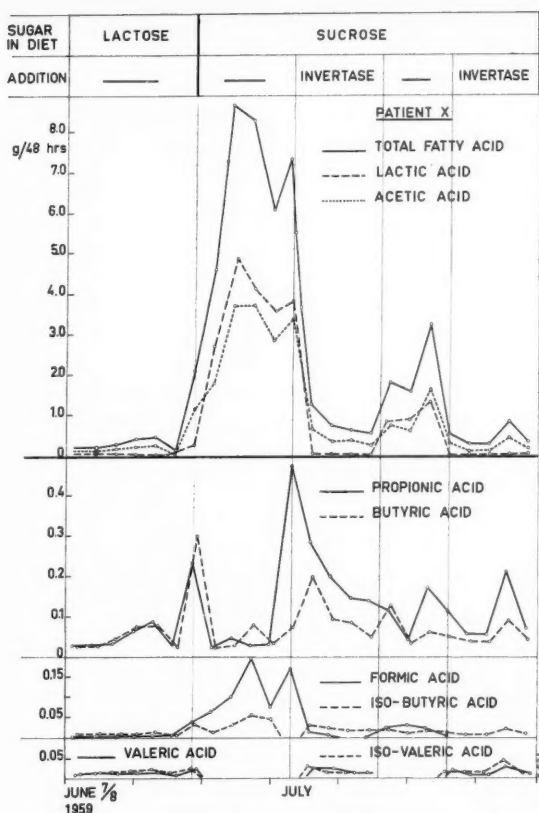


Fig. 10. Differentiation of the lower fatty acids excreted with the faeces in patient X on diets containing lactose and sucrose resp.; part of Fig. 8.

acid content, concurrently observed. Because the reliable determination of faecal streptococci in faeces is a more involved and time consuming procedure than the chemical tests mentioned it seems preferable to rely on an indirect biochemical criterion rather than on a direct bacteriological one in this case.

### Discussion

In the cases mentioned in literature (Dand 1958, 1959, 1960, Darling, Jeune)

a lactase-deficiency is causing lactosuria with or without diarrhoea, whereas in the two cases described by Holzel and coworkers diarrhoea was demonstrated without lactosuria.

In three children we were able to demonstrate that chronic diarrhoea can also be caused by maltase and/or invertase deficiencies, whereas only traces of sugar were excreted with the urine. Patient X could convert neither maltose (i.e. also starch) nor saccharose into monosaccha-

TABLE 1. *Bacteriological differentiation of faeces as influenced by diet in a patient suffering from maltase- and invertase deficiency (Mossel).<sup>1</sup>*

Bacterial counts per one gramme of fresh faeces	Diet with maltose or sucrose	Same plus corresp. enzymes; or diet with glucose only
Total aerobic count	$0.2 \times 10^{10} - 0.4 \times 10^{11}$	$0.1 \times 10^{10} - 0.8 \times 10^{10}$
Total anaerobic count	$0.7 \times 10^9 - 0.4 \times 10^{11}$	$0.1 \times 10^{10} - 0.2 \times 10^{10}$
Aerobic proteolytic count	$0.1 \times 10^5 - 0.2 \times 10^8$	$0.9 \times 10^5 - 0.5 \times 10^9$
<i>Enterobacteriaceae</i>	$0.3 \times 10^8 - 0.8 \times 10^9$	$0.8 \times 10^9 - 0.3 \times 10^{10}$
Faecal streptococci	$0.4 \times 10^9 - 0.4 \times 10^{10}$	$0.2 \times 10^7 - 0.7 \times 10^8$
Sulphite reducing clostridia	$0.4 \times 10^5 - 0.6 \times 10^7$	$0.1 \times 10^5 - 0.6 \times 10^9$
<i>Bifidobact. bifidum</i>	$0.2 \times 10^9 - 0.8 \times 10^{11}$	$0.3 \times 10^9 - 0.8 \times 10^{10}$
Yeasts	$0.6 \times 10^4 - 0.7 \times 10^7$	$0.4 \times 10^4 - 0.1 \times 10^7$
pH value of faeces	4.7-5.8	6.4-7.2

rides, while the other two little patients, though capable of converting starch into glucose, were, just as patient X, incapable of splitting saccharose into glucose + fructose. Thus the non-absorbed disaccharides and starch are attacked by the bacteria further down the intestine, which results in fermentation with subsequent formation of organic acids, mainly acetic and lactic acid. These acids, and possibly also other metabolites of the bacterial flora, irritate the intestine, which reacts to this with increased peristalsis, excretion of fluid and mucus formation, due to which the absorption is disturbed, with subsequent diarrhoea.

Of the patients discussed one therefore suffered from a *maltase + invertase* deficiency, the other two only from an *invertase* deficiency.

The invertase deficiency was excellently compensated for by an invertase preparation, and was, of course, also remedied by a saccharosefree diet.

We failed to compensate the maltase deficiency with the help of enzyme preparations, as the maltase and glucosidase preparations used were ineffective in this

respect. It may be that a combination of these two preparations, provided with a coating so that the maltase is not inactivated in the stomach, will lead to favourable results. This is under investigation at present.

Sodium bicarbonate was useless for protecting the maltase or exerting a favourable influence on its activity.

Recently we were also able to confirm in a three-months old infant with a stubborn diarrhoea the observation made by Holzel that a diarrhoea can be caused also by a lactase deficiency whereas no sugar was excreted with the urine. The diarrhoea was stopped and the baby started growing by excluding only lactose from the diet, whereas adding lactose to the diet caused again diarrhoea. The child showed also a flat lactose tolerance curve.

So far we cannot state with certainty whether these enzyme deficiencies are of a familial nature and permanent, or only transient.

Holzel and co-workers demonstrated

<sup>1</sup> The authors are greatly indebted to Dr. D. A. A. Mossel for the bacteriological investigations and for the interpretation of the results.



lactase deficiency in two children out of one family, the eldest of whom was already 10 years of age and this makes one inclined to believe that these enzyme deficiencies are familial rather than transient and this belief is strengthened by a recent observation we have made on the 13-year-old sister of patient Z, in whom we find an invertase deficiency, which had not led so far to trouble clinically as she consumes practically no sugar.

It may be concluded that deficiencies of carbohydrate splitting enzymes in the intestine may give rise to chronic diarrhoea with all the consequences involved, even with arrest of growth. This should always be kept in mind when one tries to find the cause of an intractable diarrhoea.

### Summary

In 3 children suffering from chronic diarrhoea in which all normally occurring causes (including coeliac disease) were ruled out, the absence (or defective functioning) of invertase and/or maltase was proved to be the cause. The diarrhoea was arrested with a monosaccharide diet, and in the case of invertase deficiency also an invertase preparation helped. The observation of Holzel *et al.* that a chronic diarrhoea—without excretion of lactose with the urine—also can be caused by lactase deficiency could be confirmed in another child.

The criteria were the severity of the diarrhoea, the pH of the faeces and the excretion in the stools of lactic acid and the volatile lower fatty acids, gas-chromatographically differentiated into formic acid, acetic acid, propionic acid, (iso)-butyric acid, (iso)valeric acid and caproic acid.

It appeared that for the diagnosis of diarrhoeas caused by carbohydrate intolerance, the lactic acid content of the faeces is especially valuable. Differentiation of the faecal flora gives no help.

In no case more than traces of sugar were excreted with the urine.

### Acknowledgement

We are greatly indebted to Dr. Loggers and Dr. Vogels of the Nederlandsche Gisten Spiritusfabriek of Delft, for their kindness to put the enzyme preparations at our disposal and for the interest shown in our work.

### Addendum

After delivery of this manuscript we could observe two resp. three patients in two different families suffering from lactase deficiency, giving support to the presumption that these deficiencies are ~~similar~~ *familial*?

### Experimental part

#### Faeces investigation

1. Quantity
2. Dry substance
3. pH, colorimetrically
4. Fat content according to Van de Kamer *et al.*
5. Content of lower molecular organic fatty acids according to Gerritsma

A maximum of 10 g well-mixed faeces in 50–75 ml water are transferred to a calibrated flask of 100 ml, after good trituration. After addition of 3 ml  $\text{FeCl}_3$  solution<sup>1</sup> the mixture is made up to 100 ml, following which 3 g  $\text{Ca}(\text{OH})_2$  is added. After vigorous shaking, the flask is left standing for 5 minutes; subsequently the mixture is filtered off—under suction—over a Büchner filter (ø 9 cm) provided with three discs of filter paper (Schleicher and Schüll No. 595) (Goiffon).

<sup>1</sup> Prepared as follows: a solution of 10 g  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in water, made up to 100 ml, under addition of 2 drops of concentrated HCl.



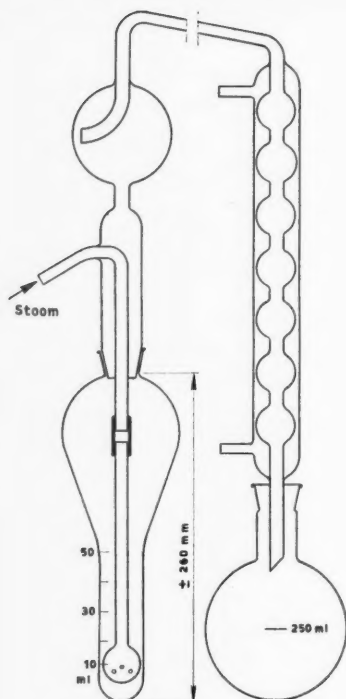


Fig. 11. Distillation apparatus according to Friedemann.

In this way the higher fatty acids are separated from the faeces in the form of Ca soaps, while the lower fatty acids are collected quantitatively in the filtrate.

#### a. Determination of lactic acid

In a part of the filtrate, containing about 1 mg of lactic acid, if need be made up to 5 ml, the lactic acid is determined according to the method of *Long* with the following modifications proposed by *Gerritsma*. Acetaldehyde is transferred to the bisulphite solution with nitrogen instead of air; crystalline potassium metabisulphite ( $K_2SO_3 \cdot SO_2$ ) is used instead of the pulverized sodium metabisulphite, while the beads used to wash the stream with bisulphite should not have a diameter larger than 3 mm.

#### b. Determination of the total content of lower volatile fatty acids

50 ml of the calcium filtrate of the faeces are distilled in a distillation apparatus according to *Friedemann* (see Fig. 11).

After addition of 8 g  $MgSO_4$  and 4 ml 16 N- $H_2SO_4$  steam is bubbled through, while the flask is heated, in the beginning with a big flame until the volume has been reduced to 30 ml.

Then the flame is adjusted in such a way that the volume remains about 30 ml, and 250 ml are distilled over in 45 minutes. After bubbling through of nitrogen, titration is done with 0.1 N NaOH on phenolphthalein ( $t_1$ ).

Then the content of lower volatile fatty acids per 100 g faeces is:

$$2 \times 10 \times 0.1 \times t_1 \text{ mEq.}$$

The content in mg of each acid separately is calculated from the titrations after gas-chromatographic separation (see sub c).

If much lactic acid is also present, a correction can be made for this, as, according to *Friedemann*, 3 % of it is carried over in the distillation.

#### c. Determination of the content of each of the lower fatty acids separately

After addition of a few drops of 0.1 N NaOH, the neutralized distillate obtained under b. is evaporated into a small volume. This is washed with water into an Erlenmeyer flask of 50 ml and dried by evaporation on a steam bath. Then are added 4 ml ether and 0.25 ml 4 N sodiumhydrosulphate. If there is more than 0.9 mEq total acid present, more sodiumhydrosulphate solution should be added, namely such a quantity that there is an excess of about 10 %. The mixture is vigorously stirred and the salt residue thoroughly loosened with a stirring rod. Subsequently, 0.5 g dry  $Na_2SO_4$  is added for each 0.25 ml sodiumhydrosulphate solution, and the solution shaken until it has become clear. After 30 minutes the solution is filtered through a small wad of cotton wool into a calibrated flask of 10 or 25 ml. The residue is eluted with portions

of 1 ml ether. After mixing with ether and making up to 10 or 25 ml, formic acid, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric

acid and caproic acid are quantitatively determined by gas-chromatography according to James & Martin modified by Van de Kamer, Gerritsma & Wansink.

### References

1. ALTHAUSEN, T. L. and UYEYAMA, K.: A new test of pancreatic function based on starch intolerance. *Ann Intern Med*, 41: 563-575, 1954.
2. ANDERSEN, D. H.: Celiac syndrome. VI. The relationship of celiac disease, starch intolerance and steatorrhea. *J Pediat*, 30: 564-582, 1947.
3. DARLING, S., MORTENSEN, O. and SONDERGAARD, G.: Lactosuria and amino-aciduria in infancy. *Acta Paediat*, 49: 281-290, 1960.
4. DURAND, P.: Lattosuria idiopatica in una paziente con diarrea cronica ed acidosi. *Minerva Paediat*, 10: 706, 1958.
5. DURAND, P.: Lactosurie et saccharosurie. *Probl Act Paediat*, 4: 496, 1959.
6. DURAND, P.: Insuffisante hydrolyse intestinale du lactose. *Pédiatrie*, 15: 407, 1960.
7. FRIEDEMANN, T. E.: The identification and quantitative determination of volatile alcohols and acids. *J Biol Chem*, 123: 161-184, 1938.
8. GERRITSMA, K. W.: Quantitative determination of the lower fatty acids in faeces by partition chromatography. Thesis Utrecht, 1954.
9. GIFFON, R.: Manuel de coprologie clinique. 5<sup>me</sup> éd. Paris, Masson et C<sup>ie</sup>, 1949.
10. HOLZEL, A., SCHWARZ, V. and SUTCLIFFE, K. W.: Defective lactose absorption causing malnutrition in infancy. *Lancet*, I: 1126-1129, 1959.
11. JAMES, A. T. and MARTIN, A. J. P.: Gas-liquid partition chromatography; the separation and micro-estimation of volatile fatty acids from formic acid to dodecanoic acid. *Biochem J*, 50: 679-690, 1952.
12. JEUNE, M., CHARRAT, A., COTTE, J., FOURNIER, P. et HERMIER, M.: Sur un cas de lactosurie congénitale. *Pédiatrie*, 15: 411-419, 1960.
13. KAMER, J. H. VAN DE, GERRITSMA, K. W. and WANSINK, E. J.: Gas-liquid partition chromatography; the separation and micro-estimation of volatile fatty acids from formic acid to dodecanoic acid. *Biochem J*, 61: 174-176, 1955.
14. LONG, C.: The stabilization and estimation of lactic acid in blood samples. *Biochem J*, 40: 27-33, 1946.
15. MOSSEL, D. A. A.: The suitability of some obligate anaerobic nonsporeforming enteric bacteria as part of a more extended bacterial association as indicator of faecal 'contamination' of foods. *J Appl Bacteriol*, 22: 184-192, 1959.
16. MOSSEL, D. A. A. and KRUGERS DAGNEAUX, E. L.: Bacteriological requirements for and bacteriological analysis of precooked ('instant') cereals and similar foods. *Antonie van Leeuwenhoek*, 25: 230-236, 1959.

Received April 4, 1960

Accepted April 22, 1960

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CASE REPORTS

## Annular Pancreas

by A. TJON SIEN KIE, P. WITTEBOL and J. LUNDING

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The occurrence of aberrant pancreatic tissue in various abdominal organs, especially in the gastro-intestinal tract, is not infrequent. The aberrant tissue is usually small, does not give rise to symptoms and is commonly found incidentally at operations and autopsies. In some cases, however, it can actually give rise to obstruction, invagination (16), or other disturbances.

In addition to mechanical complications, pancreatitis (5, 18), malignant degeneration and, occasionally, hypertrophy of the islands of Langerhans with resulting hyperinsulinism (3) occur in the aberrant tissue.

Danzis has classified ectopic pancreatic tissue as: I—aberrant pancreatic nodules, having no connection with the pancreas proper. II—annular pancreas, occurring less frequently than the nodular forms.

A combination of these types has only been reported sporadically (6).

This article is chiefly concerned with annular pancreas, with reference to two infants observed in the Pædiatric Clinic of the University of Amsterdam.

### Case I:

The patient, a male infant, the first child of young healthy parents (negative family history), was admitted to the Clinic on August 23, 1953, a few days after birth.

The birth weight was 2.700 g. When the infant received its first breast feeding, it was observed that the child did not drink well and vomited (non-projectile) a great deal; this condition remained unaltered in the course of the following days. The vomitus was egg-yellow; there was no admixture of blood. On the third day, meconium was passed. At the time of admission the child was sluggish, extremely hypotonic; the skin turgor was markedly decreased. The large fontanel was not sunken. There were mongoloid stigmata but no abnormalities were found in heart or lungs; liver and spleen were not palpable. There was, however, clearly visible peristalsis in the epigastric region.

Shortly after the first feeding, the infant vomited, in a large wave, canary-yellow fluid (acid reaction). Despite the administration of a hypodermoclysis and the discontinuation of oral feeding, the patient vomited a few more times.

On Aug. 26, roentgenological examination revealed a large gas bubble and a few smaller ones in the upper abdominal quadrant. Lipiodol, introduced into the stomach, was



Fig. 1.

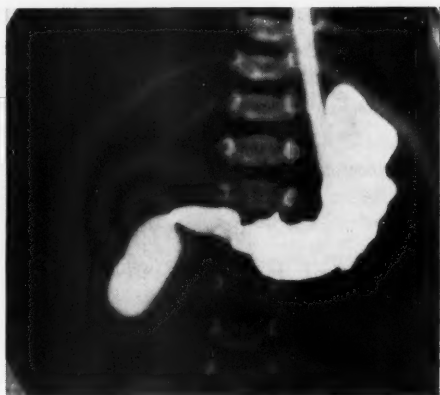


Fig. 2.

Fig. 1. Survey film of the abdomen with the presence of air in the stomach and duodenal bulb only.

Fig. 2. Lipiodol, introduced into the stomach, passed the pylorus, but was retained in the descending segment of the duodenum.

able to pass the pylorus but was retained in the last portion of the descending segment of the duodenum (Fig. 1). After four hours, all contrast media had collected in this area.

Due to the child's poor general condition operation was postponed till the next day.

At operation, it appeared that the stomach and the foremost  $\frac{2}{3}$  portion of the duodenum were markedly distended; the lumen of this latter organ showed a diameter of some four centimeters. At the border of the distended and non-distended segment, the intestine was found to be affixed to the posterior abdominal wall as well as to other intestinal loops, which accounted for the stenosis.

Directly caudal to the stricture the intestine was completely collapsed.

Cleavage of the adhesions enabled mobilization of the duodenum and it was then a simple matter to press the gaseous contents of the stomach through the stenosis. After the operation the child still vomited bile but the abdomen was no longer distended and peristalsis could be clearly heard.

On September 4, signs of abdominal distention appeared again. Roentgenologic examination demonstrated the same condi-

tion as had been present prior to the operation. In a second operation a isoperistaltic duodeno-jejunostomy was performed.<sup>4</sup> The post-operative course was good initially, but was complicated, thereafter, by dyspeptic symptoms and a fever of up to 39.4°C. The general condition deteriorated, and despite withholding of oral feeding and administration of plasma- and glucose-salt infusions, the patient expired.

Post-mortem examination revealed:

No free fluid in the abdominal cavity.

The anastomosis between the jejunum and duodenum to be adequate.

The proximal portion of the duodenum was extremely distended, and a fibrous ring was found approximately 3½ cm from the pylorus. This ring, ½ cm thick with a width of a few mm caused constriction of the duodenum leaving only a narrow opening.

Microscopic examination disclosed the presence of pancreatic tissue, containing many granulocytic inflammatory infiltrations.

#### Case 2:

The patient, a female infant born on November 28, 1958, was the first child of an elderly primiparous mother; both parents

were healthy. The gestation period was uneventful, with the exception of a toxicosis and accompanying pre-eclamptic signs at the close of the pregnancy. In the thirty-seventh week the membranes broke spontaneously and approximately eight liters of amniotic fluid were lost. A spontaneous birth followed; the child cried immediately. The birth weight was 2500 g.

On the first day of birth, it was observed that fluid ran from her mouth; there were no accompanying attacks of cyanosis. The following day, the child vomited everything (non-projectile). The vomitus was not bilious, there was no admixture of blood. Meconium was passed shortly after birth. The infant showed a continual loss of weight and became increasingly apathetic.

At admission, on December 3rd, a deathly ill, severely dehydrated, and extremely apathetic child was seen. The skin was gray-cyanotic in colour and showed practically no turgidity. There were no signs of neck stiffness; heart and lungs were normal. The abdomen was markedly sunken, there was no visible peristalsis, and palpation did not reveal any enlargement of the liver or spleen, or any abnormal resistances. Introduction of a probe into the stomach ruled out the possibility of an atresia of the oesophagus. Oral feeding was substituted by intravenous administration of plasma and glucose-salt solutions.

A great improvement in the general condition could be observed on the following day, but the infant remained, more or less, apathetic. The single vomitus that day contained haematin and showed an acid reaction; a small amount of fresh blood was also found in the stools. Investigation of the coagulation time revealed the following deviations: the prothrombin time (Quick) showed a much greater disturbance than is usually found in the neonatal period, i.e. fifty-one seconds, with a control time of fourteen seconds. The plasma-accelerator globulin concentration was found to be 16 U (normal 11–16 U); prothrombin 50 U (normal 240–300 U), proconvertin less than 5% (normal 70–120 %). Intramuscular admini-

nistration of 1 mg Konakion (vitamin K of Hoffmann-La Roche) brought the prothrombin time back to a normal value.

On December 7 the child was fed very cautiously, but due to a recurrence of vomiting, this procedure was discontinued.

December 9: another attempt at feeding was initially successful, but later on a small amount of fluid ran from the patient's mouth.

Roentgenologic examination: a survey film of the abdomen revealed the presence of air in the stomach and duodenal bulb, but there was a total absence of gas in the remainder of the abdomen. According to Clatworthy & Lloyd, the "U"-shaped form taken on by the gas in the stomach and duodenum, is typical for an obstruction of the duodenum (Fig. 2).

Operation: The transverse colon and mesocolon were adherent to the lower edge of stomach and duodenum; the intestines were in a generally collapsed condition; the colon was extremely thin.

Careful inspection of the duodenum revealed that its descending portion, lying deep in the posterior abdominal cavity, was encircled by a band of pancreatic tissue,  $\frac{2}{3}$  cm in width. Proximal to this band, the duodenum showed a marked distension. An isoperistaltic, side-to-side, duodeno-jejuno-stomy was performed. The postoperative course was complicated by bilious vomiting; however, a postoperative survey film of the abdomen demonstrated gas-filled intestinal loops.



Fig. 3. Patient at the age of six months.

The patient's general condition remained satisfactory on intravenous administration of plasma-glucose-salt solution and after 16 days the child adapted itself quite well to oral feeding.

On February 14, 1959: the child was discharged from the clinic in good condition, weighing 3500 g.

Follow-up examination on May 23, 1959, disclosed that the child no longer vomited, ate well, and had gained 3410 g (Fig. 3).

On April 7, 1960 we saw the child again; it was in perfect condition.

### Occurrence and clinical picture

Annular pancreas is a developmental anomaly in which the descending portion of the duodenum becomes encircled by a ring of pancreatic tissue.

This disturbance was first described by Tiedemann (1818) in an adult man and woman. Ecker (1862), introduced the term annular pancreas, and Vidal (1905), was the first to describe this condition in an infant; the patient being three days old. Since that time, many cases have been published (9, 13, 14, 20, 21, 23, 24, 25). The majority of the cases have been reported in males. In 1953, Swynnerton & Tanner collected sixty-four cases, fifty of whom were males. Many cases did not show any associated clinical signs and have only been reported in the literature as incidental findings after the death of the individual from other causes.

Of fifty-eight cases reported by Bickford & Williamson in 1954, only eighteen showed clinical signs.

The first symptoms usually are the result of an acute or recurrent closure of the duodenum, and can take the form of a partial or total obstruction (the two cases presented here). In the latter condi-

tion, symptoms will become manifest shortly after birth; a partial closure may become complete at a later age (Custer & Waugh: 74 yr.), e.g., oedema of the mucosa. The presence of bile pigments in the vomitus is dependent on the occurrence of the pancreatic ring proximal or distal to Vater's papilla.

Roentgenological examination with a contrast medium is generally unnecessary in a neonate with total occlusion. The presence of gas in the stomach and its absence in the abdomen, visualized on a plain survey film, usually gives substantial evidence for a diagnosis. The use of a contrast medium is, however, indispensable for the investigation of the partial closures, as manifested in older patients.

As was seen in the first patient described, an acute or chronic pancreatitis can appear, accompanied by severe abdominal pain and distension, a rapid pulse, and a rise in temperature. The inflammation can be localized in the normally present pancreas, in the aberrant pancreatic tissue ring, or in both.

In a few cases pressure on the bile ducts has resulted in the development of jaundice (1, 22). Frequently, the condition is accompanied by a gastric- or duodenal ulcer, which may give rise to haematemesis and/or melaena. The mechanism as regards this latter situation is not apparent; some investigations assume that it is due to a stasis of gastric juices. Cohen described a case where the protective mucous membrane in the pyloric area had been ulcerated; he ascribed this to an irritation by the pancreatic juices; others claim that the ulcer is due to pressure atrophy. Whether an ulcer had developed

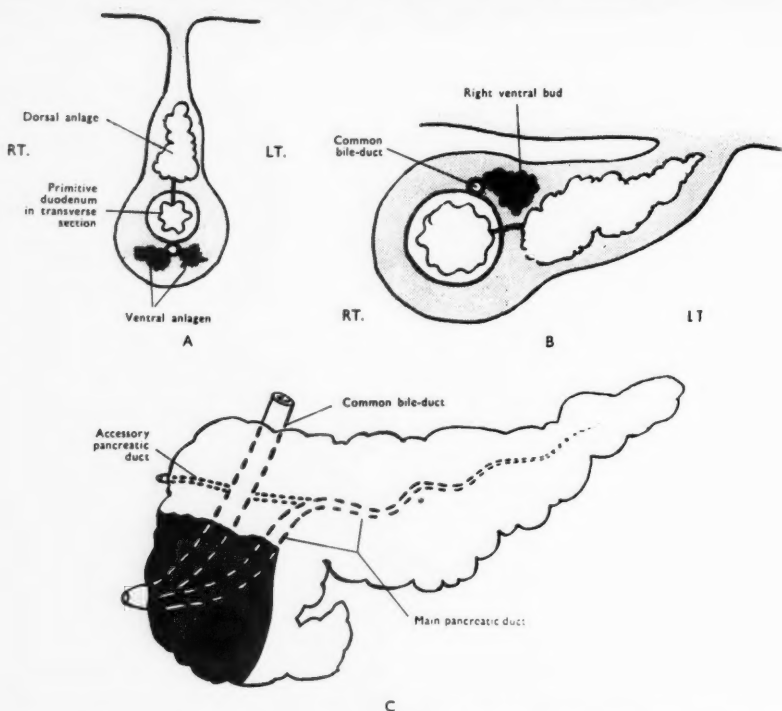


Fig. 4. Normal pancreatic development. A. Initial stage with dorsal and ventral anlage. The left ventral anlage (shaded) atrophies and disappears. B. Later stage with rotation of the duodenum. C. Adult form. (After Anderson & Wapshaw.)

in our second patient (haematin containing vomitus), cannot be ruled out with any certainty, but we are inclined to believe that this was not the case. It is most probable that the haematemesis was due to the very low concentrations of proconvertin and prothrombin. Owing to the fact that these coagulation factors were normalized after the administration of 1 mg vitamin K, it can be concluded that the deficiency of these factors was not a result of hepatic insufficiency, but rather due to a lack of vitamin K. This can be further accounted for when we consider that the bacteria, necessary for the production of vitamin K in the intestines,

were not able to reach the bowels, resulting in a disturbance in vitamin K production.

Duodenal occlusion, either due to an atresia or to annular pancreas, is frequently found in combination with other congenital abnormalities; its particular association with mongolism is well known.

#### *Aetiology:*

The pancreas is developed from a dorsal and ventral primordium (Fig. 4). The dorsal anlage lies just above the ductus choledochus and gives rise to the formation of the entire pancreas, with the exception of the undermost right quadrant of the head of this organ. The ventral anlage is made up of two seg-



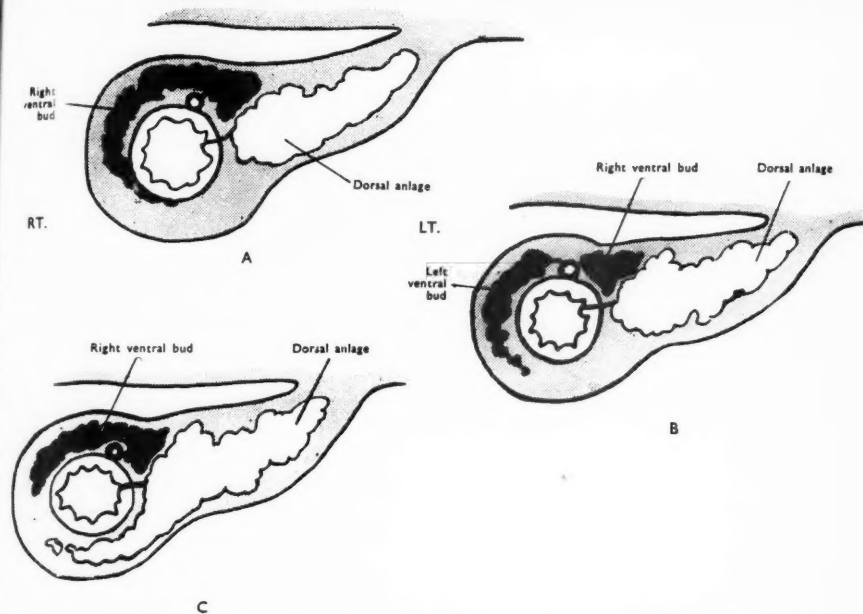


Fig. 5. Theories of development of annular pancreas. A. Lecco's theory with the fixed top of the right ventral anlage. B. Baldwin's theory in which the left ventral anlage is retained and becomes larger. C. Tieken's theory with hypertrophy of the right ventral and the dorsal anlage. (After Anderson & Wapshaw).

ments, each located on opposite sides of the ductus choledochus; the left segment usually becomes atrophic and disappears. Rotation of the intestines is accompanied by a movement of the residual ventral anlage to the right and dorsalwards, ultimately fusing with the dorsal anlage, forming the undermost right quadrant of the head of the pancreas.

At the present time, there are various theories concerning the formation of the annular pancreas (Fig. 5).

I. According to Tieken, the ring is supposed to be formed by the hypertrophy of the ventral and dorsal segments of the head of the pancreas. These two segments approach each other and fuse to the right of the duodenum. This theory is supported by Brines, and Lerat. According to Lerat, the hypertrophy is a result of an inflammatory process.

II. Lecco, assumes that the top of the

right segment of the ventral anlage becomes fixed. Due to the rotation of the intestines a band shaped organ is formed around the duodenum. This theory is in agreement with the fact that in the majority of the cases studied, the excretory duct of the ring commences on the ventral side of the duodenum, running over this organ to the right and dorsalwards, and finally coursing over the dorsal surface to the left; ultimately emptying into the duodenum.

III. Baldwin, suggests the possibility that in some cases the left segment of the ventral anlage is retained. This supposedly becomes larger in size, eventually giving rise to the formation of the ring.

IV. The potential ability to form pancreatic tissue is still retained by the primitive intestine. Normally this potential is concentrated in the dorsal and ventral anlage. If this concentration is incomplete, various

glands appear at the same level, fusing at a later stage to produce the ring-form pancreas. Erimoglu, who advanced this theory, found, after studying a tissue preparation obtained from a 46-year-old man, that in addition to the pancreatic duct, there were also four other smaller excretory ducts which ran separately from the ring to the duodenum.

### Therapy:

The necessity for surgical intervention will depend upon the degree of obstruction. In most cases, the diagnosis is not made preoperatively, and the finding of an annular pancreas usually is a surprise.

In the course of time a number of different techniques have been employed.

1. Lerat, performed a partial resection of the ring with good results, while both Silvis' and Howard's cases were complicated by a pancreatic fistula.

The frequent occurrence of duodenal atresia at the site of the pancreatic ring imposes an additional disadvantage to this technique. At most, resort can be made, in these cases, to a Heineke-Mickulicz plastic operation.

2. If the diagnosis is made at a later age, the condition is usually associated with an ulcerus ventriculi, chronic gastritis and scar formation. In these cases, Custer and Waugh resorted to partial gastric resection.

3. Posterior gastro-enterostomy was employed by Vidal, as well as by Bickford & Williamson.

4. In general, isoperistaltic duodeno-jejuno-stomy is considered to be the best technique (14). This method has the advantage that it prevents stasis in the portion of the duodenum above the obstruction.

### Summary

A description of two cases of annular pancreas in infants, which gave rise to complete obstruction of the duodenum is presented. Based on the literature and personal experience, the symptomatology, aetiology and therapy are discussed in detail.

### References

1. ANDERSON, J. and WAPSHAW, H.: Annular pancreas. *Brit J Surg*, 39: 43, 1951/1952.
2. BALDWIN, W.: A specimen of annular pancreas. *Anat Rec*, 4: 299, 1910.
3. BALLINGER, J.: Hypoglycemia from metastasizing insular carcinoma of aberrant pancreatic tissue in the liver. *Arch Pathol*, 32: 277, 1941.
4. BICKFORD, B. and LLOYD WILLIAMSON, J.: Annular pancreas, *Brit J Surg*, 39: 49, 1952.
5. BRINES, O.: Annular pancreas involved in acute haemorrhagic pancreatitis. *Ann Surg*, 92: 241, 1930.
6. CHAPMAN, J. and MOSSMAN, H.: Annular pancreas accompanied by an aberrant pancreatic nodule in the duodenum. *Amer J Surg*, 60: 286, 1943.
7. CLATWORTHY, JR. H. and LLOYD, J.: Intestinal obstruction of congenital origin. *Arch Surg*, 75: 880, 1957.
8. COHEN, H.: Accessory pancreas with ulcer of the pylorus. *Surg Gynec Obstet*, 34: 384, 1922.
9. CUNNINGHAM, G.: Annular pancreas. *Brit J Surg*, 27: 678, 1939/1940.
10. CUSTER, M. and WAUGH, J.: Annular pancreas with secondary dilatation of the duodenum. *Proc Mayo Clin*, 19: 388, 1944.
11. DANZIS, M.: Aberrant pancreatic tissue in the gastro-intestinal tract. *Surg Gynec Obstet*, 67: 520, 1938.
12. ECKER, A.: Bildungsfehler des Pankreas und des Herzens. *Z Rat Med* 14, 354, 1862.
13. ERIMOGLU, C.: A case of pancreas annulare. *Proc K Ned Akad Wet Serie C*, 55: 18, 1952.
14. GROSS, R. and CHISHOLM, T.: Annular pancreas producing duodenal obstruction. *Ann Surg*, 119: 759, 1944.
15. HOWARD, N.: Annular pancreas. *Surg Gynec Obstet*, 50: 533, 1930.
16. KITCHIN, A.: Intussusception due to ectopic pancreatic tissue. *Lancet* II, 989, 1952.
17. LECCO, T.: Zur Morphologie des Pankreas annulare. *S B Akad Wiss Wien*, 119: 391, 1910.

18. LEHMAN, E.: Annular pancreas as a clinical problem. *Ann Surg*, 115: 574, 1942.
19. LERAT, P.: Contribution chirurgicale à l'étude du pancréas annulaire. *Bull Acad Roy Med Belg*, 24: 237 en 290, 1910.
20. McNAUGHT, J.: Annular pancreas. *Amer J Med Sci*, 185: 249, 1933.
21. OTERDOOM, H.: Annular pancreas. *Ned T Geneesk*, 1: 476, 1953.
22. RAWITCH, M. and WOODS, Jr. A.: Annular pancreas. *Ann Surg*, 132: 1116, 1950.
23. RICKHAM, P.: Annular pancreas in the newborn. *Arch Dis Childh*, 29: 80, 1954.
24. SHAPIRO, D., DZURIK, F. and GERRISH, E.: Obstruction of the duodenum in the newborn infant due to annular pancreas. *Pediatrics*, 9: 764, 1952.
25. SILVIS, R.: Annular pancreas. *Ann Surg*, 135: 278, 1952.
26. SWYNNERTON, B. and TANNER, N.: Annular pancreas. *Brit Med J*, 1: 1028, 1953.
27. TIEDEMANN, F.: Über die Verschiedenheiten des Ausführungsgangs der Bauchspeicheldrüse bei dem Menschen und den Säugethieren. *Deutsch Arch Physiol*, 4: 403, 1818.
28. VIDAL, E.: Pancréas annulaire et atrophie du duodenum chez un enfant de trois jours. Gastro-entérostomie postérieure. Guérison. Dixhuitième Congrès de Chirurgie, Paris, 745, 1905.

Received March 3, 1960

Accepted April 20, 1960

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CASE REPORT

## Aortic Thrombosis and Aortic Medionecrosis in an Infant

by ANDERS MOBERG

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(Head: F. Wahlgren, M.D.)*

Aortic thrombosis—usually developing from arteriosclerosis—is, from the pathologist's point of view, a fairly common occurrence in the adult. On the other hand, however, aortic thrombosis in infancy is rare. In 1956 Moberg & Reinand studied the literature in connection with two of their own cases and since then no further cases appear to have been published. Of the 24 cases then on record where the patient was under 15, no less than 11 occurred in children less than 3 weeks old. There is no clear explanation for this preponderance of cases in the neonatal period.

The etiology of the aortic thrombosis has varied in the different cases. In some cases the thromboses have originated in the umbilical artery (10) or in the ductus arteriosus (6). In a number of cases infection is considered to have been the basic cause (3, 8, 13, 15, 19) and in one of Moberg & Reinand's cases there was medionecrosis of the aorta. In almost half of the cases, however, no definite cause has been established.

Boy born at term, weight 2420 g. The mother was a Para II and had had a previous abortion. She was healthy during pregnancy. The delivery was normal. During the first 24 hours of life the child was normal.

He subsequently became increasingly listless, vomited repeatedly and lost about 400 g daily. He was transferred to a children's surgical department where, during the sixth day of life, the entire intestines were X-rayed for suspected ileus or pylorospasm. No changes of importance were observed. The patient continued to vomit and also developed diarrhoea of a slimy consistency. During the time that the child was in hospital the pattern of symptoms changed continuously and was dominated by extreme meconium-like vomiting. Despite adequate therapy the patient remained in a shocked condition the whole time and gave the impression of being intoxicated. During the last two days of life there was fetid blood vomiting and dark brown to black excrement of a loose consistency.

Laboratory tests showed that the hemoglobin, the white blood corpuscles and the differential blood count were all of normal value. Cerebral spinal fluid examination revealed nothing abnormal. Urine examinations were normal to begin with, but on the 15th day of life numerous red and white blood corpuscles appeared in the sediment. Further blood analysis showed a slight acidosis. Despite general shock treatment the patient died on the 16th day of life.

The *post mortem* revealed no cardiac malformation. In the left auricle there was an almost pea-sized thrombus. At the inlet to the aorta the ductus arteriosus was thrombosed (Fig. 1). From this point a pea-sized

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Fig. 1. The thrombus in the ductus arteriosus extending into the aorta.

thrombus had penetrated into the aorta. Proximally this thrombus was lightly attached to the wall of the vessel.

In the abdominal aorta a large thrombus was visible (Fig. 2). This extended from the outlet of the celiac artery down to the aortic bifurcation. It did not continue into the iliac arteries. It was firmly attached to the wall

of the vessel and entirely occluded the outlets of the renal, and the superior and inferior mesenteric arteries. Both of the kidneys and a large part of the intestines showed hemorrhagic infarction.

The umbilicus was normal and showed no signs of infection. There were no thrombi in the umbilical veins. In the umbilical artery



Fig. 2. The large aortic thrombus. Note the discoloration of the kidneys due to hemorrhagic infarction.

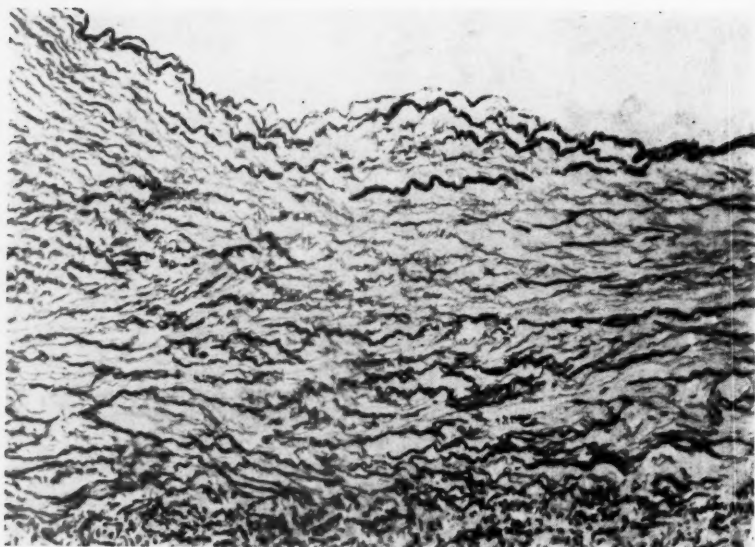


Fig. 3. Ruptures of the elastic lamellae. Weigert's elastic tissue stain. 130  $\times$  magnification.

there was a thin, 3 cm long thrombus which was lightly attached to the wall of the vessel, and which did not entirely fill the vessel.

Here and there in the lungs there were a number of almost rice-sized centres which both macroscopically and microscopically proved to be aspirated blood. No significant changes were observable in the other organs of the body.

*Microscopic investigation* revealed similar changes throughout the entire aorta. In the wall of the aorta and particularly in the media the elastic lamellae were separated by an amorphous substance with a positive mucoid colouring. Here and there the lamellae were ruptured, and in and around these areas there were strands of cell-deficient collagenous connective tissue (Fig. 3 and 4). In a few places in the aortic wall, small transverse ruptures were visible and around these ruptures a few red blood corpuscles could be seen, but there seemed to be no actual dissecting aneurysm of the aorta (Fig. 5). The ruptures must therefore have developed just before death. Direct connec-

tion between the ruptures and the aortic thrombosis has, however, not been found on the slides. There was no sign of inflammation in the aortic wall and no changes in the vasa vasorum. In the heart there were a number of very small and entirely insignificant fresh thrombi in the trabeculae of the left ventricle. The myocardium was, however, normal.

The cause of the disease and death of the patient was thus thrombosis in the abdominal aorta with secondary circulatory failure in the intestines and kidneys.

Besides the thrombus in the abdominal aorta, thrombi were also visible in the ductus arteriosus, the umbilical artery, the left auricle and in the trabeculae of the left ventricle. It is difficult to establish in which order these thrombi developed. From the circulatory point of view it would seem to have been the thrombi in the heart that were the primary ones. But the fresh appearance of these belied this, apart from the fact that neither macroscopically nor microscopically could any reasons be found as to

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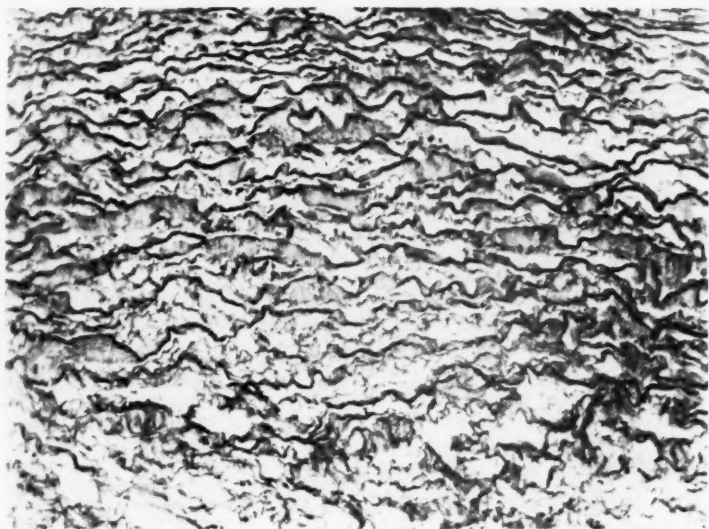


Fig. 4. Detail enlargement of Fig. 3 (see text). Weigert's elastic tissue stain, 250  $\times$  magnification.

why the thromboses should have developed in these positions. It is more likely that the thrombus in the ductus arteriosus was the primary one and that fragments of this had broken loose, fastened in the aortic bifurcation and here developed into the large thrombus. Similar cases of this type have been described previously (6). Both the macroscopic and microscopic pictures, however, indicated that the thrombus in the abdominal aorta was older than the one in the ductus arteriosus. The microscopic investigation of the aorta revealed, in several places, changes in the media and specially within the elastic tissue. These changes correspond with those that are usually seen in aortic medionecrosis. Transverse ruptures were observed, although not directly connected with the thrombus. Despite this it seems probable that these ruptures, the aortic medionecrosis and the aortic thrombosis, were directly connected. Similar cases of this type have also been described in the literature (11).

It thus seems likely that the primary changes were aortic medionecrosis with

secondary thrombosis in the abdominal aorta. It is generally known that thromboses can develop during the period of closure of the ductus arteriosus and the

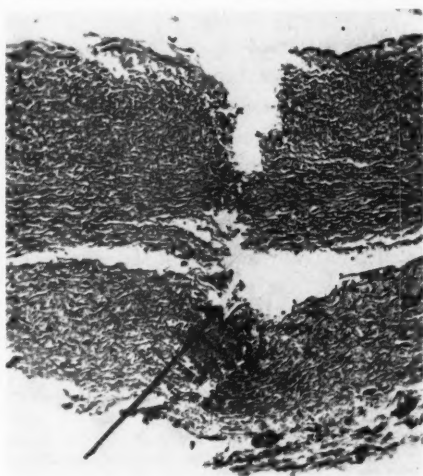


Fig. 5. Transverse rupture. The arrow indicates a few red blood corpuscles. Ladewig modification of the Mallory stain, 150  $\times$  magnification.



umbilical artery. It seems probable that this is what happened in these vessels. This was macroscopically evident at least in the case of the umbilical artery. It is difficult to explain the minor thrombi in the heart. It might possibly have been a result of a coagulation disorder, but unfortunately the blood coagulation properties were not investigated. Another and more likely explanation is hemoconcentration, since the patient died in a dehydrated condition.

### Discussion

Medionecrosis aortae is a well-known disease in the adult. Gore & Seiwert have reported 32 cases of dissecting aortic aneurysm in patients of less than 40 years of age. They found patchy necrosis of the elastic substance in the media. These changes agree with those which i.a. Merz has described in aortic medionecrosis in infants.

The etiology of this disease, which in itself is a causation of dissecting aortic aneurysms, has been widely discussed. Certain authors (2, 12, 18,) have found changes in the vasa vasorum. They conclude that ischemia of the media is the reason for the medionecrosis. Other authors (4, 17), however, have not been able to demonstrate changes in the vasa vasorum. Gore has put forward the theory that aortic medionecrosis is of congenital

origin on a biochemical or metabolic basis. This theory is supported by the fact that Abbot, Gore, Reifenstein *et al.* have found a high percentage of congenital anomalies in the heart or aorta in these cases. Holle, Wolf; Moberg & Reinand (Case 2) have all previously described cases of aortic medionecrosis in infants less than 3 weeks old. These cases and the case described here indicate that aortic medionecrosis occurs in the neonatal period. This strongly supports the theory of the congenital origin of the disease.

### Summary

In the literature 24 cases of aortic thrombosis in children have been described of which 11 patients have been less than 3 weeks old. A case is described with a large thrombus in the abdominal aorta of a 16 day old boy. Thrombi were also discovered in the ductus arteriosus, the umbilical artery, the left ventricle and the left auricle. Histological examination of the aortic wall revealed changes of the type that are usually seen in aortic medionecrosis. It is presumed that in accordance with similar cases of aortic medionecrosis the thrombosis and the medionecrosis are directly connected. The etiology is discussed. This case, like a few previously published cases of aortic medionecrosis in the neonatal period, indicates that the disease is of a congenital origin.

### References

1. ABBOT, M. E.: Coarctation of aorta of adult type; statistical study and historical retrospect of 200 recorded cases with autopsy of stenosis or obliteration of descending arch in subjects above age of 2 years. *Amer Heart J*, 3: 574, 1928.
2. AMROMIN, G. D., SCHLICHTER, J. G. and SOLWAY, A. J. L.: Medionecrosis of the aorta. *Arch Path*, 46: 380, 1948.
3. BOBEFF, D.: Embolie der Bauchaorta nach Diphtherie. *Mösch Kinderhk*, 71: 69, 1937.

4. GORE, I.: Dissecting aneurysms of the aorta in persons under 40 years of age. *Arch Path*, 55: 1, 1953.
5. GORE, I. and SEIWERT, V. J.: Dissecting aneurysms of the aorta. *Arch Path*, 53: 121, 1952.
6. GROSS, R.: Arterial embolism and thrombosis in infancy. *Amer J Dis Child*, 70: 61, 1945.
7. HOLLE, G.: Über mehrfache angeborene mesenchymale Defektbildungen mit Medionecrosis aortae bei einem 3 Monaten alten Säugling. *Z Ges inn Med*, 1-2: 502, 1946-47.
8. MENASCI, R.: Aortic thrombosis complicating influenza. *Riforma Med*, 43: 149, 1927.
9. MERZ, W.: Über mehrfache und unvollständige Rupturen der Aorta ascendens und des Arcus aortae. *Frankfurt Z Path*, 59: 267, 1947-48.
10. MIETTINEN, M.: On thrombosis in children. *Acta Pædiat (Upps)*, 39: 267, 1950.
11. MOBERG, A. and REINAND, T.: Aortic thrombosis in infancy. Two cases of different etiology. *Acta Path Microbiol Scand*, 39: 161, 1956.
12. MORITZ, A. R.: Medionecrosis aortae idiopathica cystica. *Amer J Path*, 8: 717, 1932.
13. MOSCHOWITZ, E.: Thrombosis of aorta and both iliac arteries following streptococcus infection of the umbilical cord. *Proc New York Path Soc*, 14: 21, 1914 cited by Rothstein.
14. REIFENSTEIN, G. H., LEVINE, S. A. and GROSS, R. E.: Coarctation of aorta; review of 104 autopsied cases of "adult type" 2 years of age or older. *Amer Heart J*, 33: 146, 1947.
15. ROTHSTEIN, J. L.: Embolism and thrombosis of the abdominal aorta in infancy and in childhood. *Amer J Dis Child*, 49: 1578, 1935.
16. ROTTINO, A.: Medial degeneration in non-ruptured aorta appearing syphilitic macroscopically. *Arch Path*, 27: 320, 1939.—Medial degeneration of aorta as seen in 12 cases of dissecting aneurysm. *Ibid*. 28: 1, 1939.
17. SAILER, S.: Dissecting aneurysm of aorta. *Arch Path*, 33: 704, 1942.
18. TYSON, M. D.: Dissecting aneurysms. *Amer J Path*, 7: 581, 1931.
19. WHEELER, E.: Thrombosis of aorta and iliac arteries following pneumococcal infection of umbilicus. *Canad Med Ass J*, 11: 532, 1921 cited by Rothstein.
20. WOLFF, K.: Unbekannte Erkrankung der Säuglingsaorta mit Schwund des elastischen Gewebes. *Virchow Arch Path Anat*, 285: 367, 1932.

Received April 19, 1960

Accepted June 2, 1960

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PROCEEDINGS OF PEDIATRIC SOCIETIES

Danish Paediatric Society

Meeting January 13, 1960

**J. Vesterdal:** The Effect of Durabolin (19 nor-androsterolone) in a Case of Hypophyseal Dwarfing

The patient was a boy aged 10 years who had suffered from eosinophile granuloma from the age of two years. The disease gradually subsided but the following sequelae persisted: deaf-mutism, diabetes insipidus, adiposity and dwarfism presumably of hypophyseal origin. The boy was treated for 12 months with Durabolin (12.5 mg intramuscularly per week) during which time the rate of growth increased 9 cm compared with 4 cm the preceding year and 2 cm in the subsequent eight months. Marked increase in weight but only insignificant virilization were observed as side-effects. No definite acceleration of the development of the centres of ossification was observed.

DISCUSSION. *E. Thamdrup:* May the increase in growth perhaps be an effect of testosterone? Dwarfism is treated, as a rule,

with preparations of testosterone commencing at puberty. — *Henning Andersen:* A human growth hormone has been manufactured and appears to be effective but is not yet on the market. Durabolin is scarcely more growth-promoting than testosterone. The employment of Durabolin in premature and cachectic children is not advised, because experiments on rabbits suggest that the preparation may have a sterilizing effect on females. — *P. Plum:* Physex (20 injections of 1500 units) produces increased growth and simultaneous commencement of puberty in some cases.

**B. Friis-Hansen:** Anaemia, Malnutrition and Parasitic Infestations in African Children

(To be published elsewhere.)

**B. Friis-Hansen:** Vitamin A Deficiency and Blindness in African Children

(To be published elsewhere.)

Meeting February 10, 1960

**Sr. Brandt:** Demonstration of Generalized Muscular Hypoplasia and Bulbar Paresis in an Eight-year-old Boy

A boy aged eight years was demonstrated, who since birth, has suffered from generalized muscular hypoplasia. The condition resembled muscular dystrophy clinically but there had been no evidence of progression. In addition, the boy showed signs of hypoplasia and weakness corresponding to the

muscles innervated by cranial nerves VII, IX, X, XI and XII. Paralysis of the soft palate had been present since birth and had caused serious feeding difficulties so that the boy spent the first 2½ years of his life in paediatric wards where he had to be tube fed for long periods. At present, there are no feeding difficulties but speech is still affected by the paralysis of the soft palate and incompetence of the labial muscles. This

combination of congenital hypoplasia of muscles with bulbar innervation (nuclear hypoplasia?) and generalized muscular hypoplasia is very rare. On the other hand, both conditions occurring separately are well known although also rare. Myasthenia gravis was excluded by a prostigmine test when the child was six months old. Muscular dystrophy appears to have been excluded by a biopsy from the vastus lateralis when the boy was 14 months old. Electromyography, when 15 months old, showed short potentials (deltoid, tibialis anterior) and a tendency to fall-out in potential. (Short duration of potential is observed in polymyositis and in non-hereditary conditions which resemble muscular dystrophies. Both of these conditions appear improbable in the present case on account of the course and the biopsy findings.)

#### *H. Dyggve: Congenital Ascites*

Six cases of congenital ascites accompanied by generalized oedema and hydramnios but without blood type incompatibility are reported. The sixth case, a male infant aged two months, is still in the Paediatric Department of the University Hospital. He probably has chylous ascites. On the second, eight and thirtieth days of life, approximately 400 ml ascitic fluid were withdrawn by abdominal paracentesis. This fluid became increasingly milky and contained 0.5 per cent fat. A cystic swelling could be felt at the site of the gall bladder. When the infant was five weeks old a cyst of the liver containing chocolate-coloured fluid was removed at exploratory laparotomy. Ascites has again accumulated. The treatment planned is repeated abdominal paracentesis and a low-fat, protein-rich diet. If this proves ineffective, veno-peritoneal anastomosis (Routte's operation) may be considered. Thirteen cases of congenital chylous ascites which were cured by other operations have been described while only two died.

**DISCUSSION.** *J. Flamand Christensen:* In the case from Odense, no thoracic duct was found and many of the lymph vessels in the

intestine ended blindly. Other operations have also been attempted and among these, isolation of a small segment of the small intestine which is opened and sewn to the peritoneum has been effective.

#### *J. H. Probst: Vitamin D-Resistant Rickets*

A boy aged 14 years suffering from vitamin D-resistant rickets was followed for 12 years. The findings deviate from those accepted as typical in the somewhat early commencement (at the end of the first year of life), only slightly lowered or sometimes low to normal serum phosphorus and very low serum calcium (as low as 5.4 mg/100 ml). The alkaline phosphatase values were elevated and were normalized by adequate therapy. The dosage of vitamin D necessary was between 50,000 and 250,000 units daily. Hypercalcaemia (12.9 and 14.2 mg/100 ml) occurred for two brief periods. The symptomatology of this complication is discussed: polyuria, polydipsia, reduced renal concentration, slight azotaemia and slight sporadic pyuria and uncharacteristic cerebral seizures with slightly abnormal EEG, all of which were completely reversible on normalization of the serum calcium by reduction of the dose of vitamin D. Normalization of the alkaline phosphatase values together with healing of the bone lesions and increased growth in height are considered to be the best indications of the efficacy of the treatment. Hypercalcaemia is an indication of overdosage and raised alkaline phosphatase values for underdosage. The occurrence of hypercalciuria prior to hypercalcaemia makes Sulkowitz's test a valuable control measure. The diminishing requirement of vitamin D with increasing age makes increased control necessary at puberty when overdosage may easily occur. Corrective osteotomy should only be undertaken late and preferably when the patient is fully grown; it is useless before normalization of the bone lesion. There is a risk of hypercalcaemia following the osteotomy and, for this reason, vitamin D should be temporarily withdrawn during this period. Hypophosphataemia is regarded as a more sensitive indicator of the presence of the abnormal

gene than a manifest skeletal lesion. The mode of inheritance of the hereditary cases (approximately half of the cases recorded in the literature) is considered to be a sex-linked dominant. Female carriers only rarely show bone manifestations and, if so, the changes are minimal. The sporadic form, to which the present case belongs, cannot be differentiated metabolically from the hereditary form but, in this form, females may also have severe skeletal involvement. Current opinions concerning the pathogenesis are discussed.

**DISCUSSION.** *Dr. Brandt:* In orthopedic departments, surgical intervention is frequently undertaken on severely deformed bones. To what extent can growth correct the deformities so that surgical intervention may be avoided? — *J. Flømand Christensen* had followed a similar case in a boy who had a serum calcium of 6.8 mg/100 ml at the age of two years and who had been hospitalized a year previously elsewhere on account of seizures. The serum phosphorus was not less than 4 mg/100 ml and the phosphatase 40 King-Armstrong units. Slight acidosis was present. Increased excretion of cystine and lysine in the urine were found on repeated amino acid investigations. The child improved on treatment with 60,000 units of vitamin D daily with supplementary calcium and phosphorus. An uncle possibly also had the disease. — *B. Friis-Hansen:* Bone deformities may be due to many other diseases and, therefore, medical treatment should be

attempted first before surgical intervention is resorted to. — *Henning Andersen:* It is surprising how much bone deformity may improve between the ages of 12 and 15 years and therefore osteotomy should not be undertaken prior to puberty except under special circumstances. — *J. H. Probst:* It is uncertain whether the sporadic and the familial cases are the same disease. Osteotomy should be undertaken only during periods of biochemical homeostasis and may then be of good effect. Otherwise, patience must be exercised.

***Bjørn Andersen & Folke Tudvad: Attempts to Determine the Enzymatic Activity of the Intestinal Canal under Physiological Conditions***

In the Children's Hospital, Fuglebakken, Copenhagen, attempts were made to measure the enzymatic activity in the intestinal canal particularly in children with dyspepsia. Short plastic tubes of suitable diameter containing either protein or fat were administered orally to a total of 22 patients. The tubes were recovered from the faeces and the amount of the substrate digested measured. Eleven children without or with only insignificant transient dyspeptic symptoms digested, on an average, 2.4 mg protein and 2.0 mg fat, while 11 children with dyspepsia of longer duration showed much greater digestion both of the protein and particularly of the fat. This returned to normal as the dyspepsia improved.

**Meeting March 9, 1960**

***M. Osler: The Body Composition of New-born Infants of Diabetic Mothers***

The body composition of new-born infants of diabetic mothers was determined on the basis of investigation of the fluid content, the fat content, fluid distribution in intra- and extracellular spaces and neonatal deviations in the fluid content, the conditions of excretion in the urine and the maturity as determined by the development of the centres of ossification. The conclusions drawn

from the investigations were that infants born to diabetic mothers must be described as infants whose maturity, as expressed by the development of the centres of ossification, water and nitrogen excretion in the urine and the functions of the other organs, corresponds to their chronological age or perhaps slightly less, i.e. they are premature. In addition, they are markedly overweight because of excessive fat and, to a lesser degree, carbohydrate depots in the tissues and organs.

The incomplete development of the function of the organs, fluid release on the breakdown of the carbohydrate depots following birth and adiposity which impedes respiration are considered to be of possible significance for the frequent occurrence of the neonatal pulmonary syndrome in infants born to diabetic mothers. Insulin, particularly in combination with copious administration of carbohydrate, increases the fat and carbohydrate depots without influencing the maturity, whereas growth hormone reduces the fat content. It must therefore be presumed that the macrosomia of diabetic children is due to increased supply of glucose from the mother with subsequent increased insulin production in the foetal pancreas.

**DISCUSSION.** *B. Friis-Hansen:* We probably harm these infants by putting them in incubators with relatively high humidity which impedes their fluid expenditure. — *H. Dygge:* It has been stated in the literature that the incidence of the hyaline membrane syndrome is reduced when glucose is administered. If infants develop respiratory difficulties, they must have oxygen but attention must be paid to the degree of humidity and the body temperature should not be reduced too much. The target should be 35–36°C. — *M. Osler:* The time of delivery should be adjusted, bearing in mind the facts that full-term births result in more deaths *in utero* while too premature births result in more cases of the respiratory distress syndrome. The great nitrogen excretion is due to breakdown of protein. Seizures were described in approximately half of the infants and these were probably due to low serum calcium. By and large, the excretion of electrolytes was great. Excretion of calcium may perhaps increase the excretion of potassium. — *Nygen Pedersen:* We have demonstrated that these infants do not have hypoglycemia and no definite effect could be demonstrated from administration of glucose. Care should probably be exercised in the administration of fluids. One third of the diabetic mothers were delivered by Caesarean section with a low infantile mortality.

### *J. H. Probst: Two Cases of Water Intoxication*

**CASE 1.** The patient was a boy aged four months with congenital megacolon. The infant was admitted in a state of marked dehydration (serum Na 164 mEq/l), unconscious and with a distended abdomen following gastroenteritis. Despite careful rehydration and repeated lavage of the intestine in the course of the night with transient improvement, the infant developed an attack of generalized prolonged tonic seizures 18 hours after admission. The child was comatose with bilateral Babinski reactions; stimulation by touch triggered the seizures. The cerebrospinal fluid, serum calcium and bicarbonate were normal. It was then discovered that the intestinal lavage had been undertaken with tap water and that the return was negligible. The serum Na was 131 mEq/l and the total osmotic concentration 270 mOsm/l. One hour after administration of hypertonic mannitol, complete clinical remission occurred despite falling sodium values but increasing osmotic pressure. The infant smiled, could fixate and suck and the plantar reflex was normal. The improvement continued and, following a massive diuresis, the serum values returned to normal. EEGs recorded one and seven months later were normal.

**CASE 2.** The patient was a boy aged four years with a fractured pelvis and slight cerebral injuries following a traffic accident. During the first day there was pronounced oliguria with a specific gravity of 1.022 and catheterization was necessary. Gastric suction was instituted on account of vomiting and abdominal distension. Intravenous therapy was instituted on the fourth day. Five per cent glucose solution was administered in quantities of 1200 (+750 orally) and 1950 ml on the fourth and fifth days. Generalized tonic-clonic seizures occurred suddenly in the middle of the fifth day. The Babinski reaction was present and the child lost consciousness. Thereafter, numerous seizures followed. Examination of the eye grounds was normal. The CSF was also normal.



Serum chloride was 75 mEq/l. An unsuccessful attempt was made to control the seizures with massive intravenous administration of chlorpromazine, nembutal and pentothal. Respiratory difficulties necessitated tracheotomy on the fifth day. Shortly before transfer to the University Hospital the intravenous fluid therapy was changed to glucose-saline. This was continued as saline and the barbiturates were withdrawn. There were no further seizures and the diuresis in the subsequent 24 hours was 1750 ml and the boy's condition improved. During the subsequent three days, he showed a total negative fluid balance of over 2000 ml corresponding to 18 per cent of the body weight. The serum values returned to normal.

In addition to the diagnostic difficulties in a case of seizures in a child with the possibility of cerebral injury, the explanation here is probably that such an overdosage of water (5 % glucose) occurred because the fluid balance was recorded on a chart for adults. This chart had a printed value for fluid loss by perspiration of 1500 ml while the calculated value for the boy was 560 ml and thus an excessive dosage of one litre was administered. The antidiuresis was primarily post-traumatic and later probably maintained by the massive doses of barbiturates which prevented possible homeostatic self-regulation. Replacement of the loss of electrolyte-containing fluids by 5 % glucose solution further accentuated the hypotonicity.

Crawford, Dodge & Probst demonstrated by experimental studies on rabbits with balance experiments, EEG and brain analyses that the essential element in water intoxication is hypotonicity of the body fluids with subsequent intracellular oedema. The symptoms of water intoxication are neurological, as the brain cells are particularly sensitive to oedema and perhaps mostly because they are enclosed in the rigid cranium. Damaged cells are particularly vulnerable. Frequently seizures occur with a too rapid return to normal values. The clinical symptoms of water intoxication include increasing clouding of consciousness, adynamia and apathy and the patients are fre-

quently pale, cold and sweating ("pseudo-shock"), but the blood pressure and pulse are normal until terminally. Great quantities of dilute urine are voided. Spasms and tremor occur and the Babinski reaction is present. Later, generalized tonic-clonic seizures occur. Barbiturates have a transient effect. The coma becomes deeper and ends fatally in the untreated cases. The criteria for hypotonicity are the low serum electrolyte values or, even better, reduced serum osmotic concentration. The EEG shows greatly reduced amplitude, practically absent normal rhythm and dominance of abnormal slow frequencies. Treatment consists of administration of a hypertonic solution: 3 % sodium chloride, 15 % mannitol or 6 % urea with 5 % glucose, with the object of increasing the tonicity by approximately 20 mOsm/l in the total body fluids. By choosing the above-mentioned fluids, all of which remain extracellularly, the correct fluid distribution is ensured with resulting dehydration of the cells. In the rabbit experiments and in Case No. 1, it was demonstrated that improvement occurred during mannitol therapy, although the sodium and chloride values in the serum continued to fall, thus proving that the tonicity is the decisive factor. In milder cases, particularly when no serious cerebral symptoms demand immediate therapy, the patient may be made to dehydrate himself by reduction of the amount of fluid administered.

**DISCUSSION.** *B. Friis-Hansen:* During the days immediately after cardiac operations, patients have a tendency to tremor. In two cases my colleagues and I found hyponatraemia which we interpreted as water intoxication. Fifty per cent glucose solution administered intravenously produces an almost immediate effect on oedema of the brain. — *P. W. Braestrup:* Water intoxication is probably much more common than is assumed. I have also seen a case in a child who had just undergone a cardiac operation and who had EEG changes and seizures. — *Henning Andersen:* Lavage of a megacolon with isotonic saline is not entirely satisfactory either. We now employ 7 % gelatin solution. —

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*H. J. Probst:* The mechanism of the development of the symptoms in cardiac patients is unknown; it may be due to sodium loss. The clinical picture corresponds to that encountered in water intoxication and many of the cases described as shock may have been cases of water intoxication. The symptoms may develop in the course of 20 minutes. Gelatine solution is excellent but cannot be employed in the presence of barium. Saline lavage may be undertaken once or twice without untoward results.

***Poul Anthonisen, O. Steinicke & Aage Thomsen: A Case of Thrombocytopenic Purpura Complicating Infectious Mononucleosis***

After a few days with symptoms suggestive of acute leucosis with swelling of lymph glands, enlargement of the spleen, epistaxis and haemorrhage from the mouth, a boy aged seven years developed pronounced purpura of the skin and macroscopic haematuria. The increased haemorrhagic tendency was caused by a low thrombocyte count in the blood

(minimum 3000/mm<sup>3</sup>). The haemorrhages were so massive that signs of impending circulatory shock developed. This was corrected with blood transfusions. One week after the onset of the illness, the boy developed violent tonsillitis with membrane formation. Blood smears at this time and retrospective examination of previous preparations revealed the characteristic picture of mononucleosis. Evidence of liver involvement (raised thymol reaction, serum transaminase and high gamma globulin values) was also present. The Paul-Bunnell reaction was negative or doubtfully positive, but this is not evidence against the diagnosis. After the elapse of three weeks, the boy felt quite well again.

Review of the literature revealed only 26 corresponding cases. The cause of thrombocytopenia in mononucleosis is unknown. The case described was treated with prednisone, although, according to the literature, there is no evidence that steroid treatment has any effect. The prognosis is good.

Meeting April 20, 1960

*F. Tudvad* paid tribute to the memory of the late Dr Axel Friedländer.

***H. Kreutzfeldt: Continued Experience with Triple Vaccine***

The results of experiments with prophylactic vaccination with triple vaccine (diphtheria-tetanus-pertussis vaccine) undertaken on 655 children in a series of institutions are presented. The protection against whooping cough was satisfactory and complications connected with the vaccination do not appear to have been greater in extent than those which may occur following vaccination with diphtheria-tetanus vaccine. It is therefore recommended that generalized prophylactic vaccination with triple vaccine be introduced from the age of 4-5 months. In addition, the indications and possibilities for the introduction of vaccination with pertussis vaccine alone are discussed.

***Sv. Tulinius: Experience with Pure Pertussis Vaccine***

Three doctors, in co-operation with the National Serum Institute administered pertussis vaccine prophylactically to the infants in four infant nurseries and three child-welfare centres. In the child-welfare centres triple vaccine was occasionally employed after the age of five months. In the nurseries triple vaccine was only employed in a few cases.

Of 431 children in the four nurseries vaccinated against pertussis, 65 were exposed to infection prior to the age of five months and only one of them contracted the disease. The material from the child-welfare centres does not permit evaluation of the effect of vaccination as the exposure is unknown and it is not known with certainty how many developed whooping cough.

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and induration occurred at the site of vaccination and attained a maximum in the course of 6-7 hours and many of the infants were restless and fretful for a couple of hours about this time. No high temperatures were recorded. In isolated cases, the mothers stated that the children were irritable for a couple of days. No abscesses at the injection site nor generalized hypersensitive reactions occurred.

It is concluded that pure pertussis vaccine may be employed with advantage from the age of five weeks, that the side-effects are minimal and the effect is apparently good.

**DISCUSSION.** *H. Kreutzfeldt:* Prophylactic vaccination with triple vaccine may probably be carried out within the existing legal programme with the amendment that an extra vaccination must be given. On the other hand, free vaccination with pure pertussis vaccine would require a special paragraph in the law. The National Serum Institute and the Ministry of Health might consider that an attempt be made to carry out free vaccination with triple vaccine but to run parallel studies with pure pertussis vaccine in three groups of children: (1) those exposed to infection to determine therapeutic effects; (2) institutionalized children under the age of four years; (3) other children under the age of four months at the parents' request. It would be desirable if the National Health Insurance could pay for vaccination for group (1), the Ministry for Social Affairs for vaccination for group (2), while those who seek vaccination in group (3) must defray the cost themselves. — *Sv. Tulinius:* Triple vaccination cannot be commenced prior to the age of five months as the effect of diphtheria-tetanus vaccination is not otherwise achieved. I am therefore in favour of the programme outlined by Dr Kreutzfeldt but would not recommend booster injections, as it is desirable that as many children as possible have a mild attack of whooping cough in childhood. Booster injections may delay whooping cough until adult life. — *P. W. Bræstrup:* It would be desirable to carry out early pertussis vaccination in all children

but the practical difficulties, including the establishment of new laws, are probably too great. In addition, there is a risk that people, as a whole, become tired of vaccination and that it becomes difficult to record all the various vaccinations. — *J. Flamaud Christensen:* What would happen if a single pertussis vaccination was administered at the prophylactic health examination at the age of five weeks followed by triple vaccine at the age of five and six months? Can polio vaccination administered simultaneously with triple vaccination be expected to produce the same good antibody formation as previously? — *P. Plum:* Will whooping cough not disappear as vaccination is gradually carried out so that there is no longer such a danger for new-born infants? — *C. Friderichsen:* Is it possible that new-born infants who, for example, are exposed to whooping cough in their homes can be protected with four or five injections at short intervals? Can pertussis vaccination be continued in a child who has had convulsions in connection with vaccination? — *Sv. Tulinius:* It is scarcely correct to give pertussis vaccination alone at the age of five weeks and continue with triple vaccine at the age of five and six months. A good increase in the antibody titre for pertussis would probably occur but there is a risk that the increase in the diphtheria and tetanus antibodies is poorer. Polio vaccination may well be administered simultaneously with triple vaccination. It is scarcely probable that whooping cough will disappear even when vaccination has been carried out. A number of mild cases of the disease will probably still occur which are, however, just as infectious. Protection may be anticipated by pertussis vaccination of new-born infants, which, however, rapidly disappears. — *K. Wilken-Jensen:* Experience in vaccination of asthmatic patients does not suggest that more antigens reduce the antibody formation for one or more of the antigens. — *I. Scheibel:* Triple vaccine should not be administered prior to the age of four to five months as too many infants have too many antibodies in the blood as a result of diphtheria vaccination in the 10-

ther. These are not excreted until after approximately 20 weeks of age and thus the full effect of vaccination in the infant cannot be expected until after this time. — *F. Bruhn-Petersen*: Has any attempt been made to protect infants from whooping cough by vaccinating the mothers during pregnancy? — *Sr. Tulinius*: As far as I know, this has never been attempted and, personally, I would not recommend it on account of the risk of marked reactions in the pregnant woman and possible injury to the foetus. — *Ane Madsen*: At the child-welfare centres only a few mothers did not desire pertussis vaccination. — *Torben Iversen*: Does the National Serum Institute still recommend therapeutic vaccination (several injections at short intervals) to children exposed to whooping cough? — *Sr. Tulinius*: The National Serum Institute no longer recommends this form of vaccination. It must also be pointed out that the Institute cannot at present supply triple vaccine for purposes other than the experimental work at present in progress. At least eight months' preparation will be required before the vaccine is generally available. — *F. Tudvad*: As there seems to be unanimity as regards the desirability of altering the hitherto employed diphtheria-tetanus vaccine to diphtheria-tetanus-pertussis vaccine, the Danish Paediatric Society will appeal to the Ministry of Health in this respect and recommend that permission be given for administration of four vaccinations free of charge.

#### *Bjorn Andersen & Kjeld Nielsen: Serum Proteins in Premature Infants*

In the Children's Hospital, Fuglebakken, the variations in both the total serum proteins and the various fractions, were investigated in 39 premature infants who did not present any clinical symptoms apart from prematurity. The birth weights ranged from 1200 to 2500 g. Both longitudinal and group investigations were undertaken and the results were in agreement. Normal curves for the absolute values of total protein, albumin and gamma globulin were established in group investigations of premature infants

fed exclusively on breast milk. The total protein and albumin values fall from the first day of life until the age of five to six weeks and thereafter rise again until the age of ten weeks. The gamma globulin values fall steadily during the first ten weeks.

In group investigations in premature infants who had received the same quantities of breast milk plus supplementary protein in the form of 1½ % casein-hydrolysate (Idon), increased values for albumin and total protein were observed during the first ten weeks, although this group experienced the same fall as premature infants fed on breast milk alone. On the other hand, reduced gamma globulin values were found throughout the same period. It thus appears that supplementary protein added to breast milk with the object of accelerating growth produces increased total protein and albumin in the serum but decreased gamma globulin values.

#### *Kjeld Nielsen: Two Cases of Tumours of the Lung in Children*

Two cases of tumours of the lung admitted to the Children's Hospital, Fuglebakken, within a year are presented.

The first case was a girl aged 10½ years who had suffered from pyrexia and slight cough for more than three weeks prior to admission in February 1959. X-ray of the lungs showed infiltration superiorly in the right lung with enlargement of the hilar glands. This remained stationary for three months. Tomography, which raised the suspicion of pulmonary cysts, revealed that the infiltrations were situated posteriorly in the right lung. There was a positive tuberculin reaction following previous Calmette vaccination. Culture from the stomach washings proved negative for tuberculosis. The blood sedimentation rate was initially elevated but later returned to normal. The cold agglutinin and ornithosis serum reactions were negative as were the skin tests for fungus disease. Because the diagnosis was uncertain, the girl was treated with antituberculous chemotherapy for a month after transfer to the Department of Thoracic Surgery in Øresundshospitalet. At subsequent operation, an

elongated hard tumour the size of a Brazil nut was found extending towards the right hilus. Microscopic investigation revealed mesodermal cellular undifferentiated tumor tissue, probably a neurofibrosarcoma.

The second case was a girl aged four years who had suffered from uncharacteristic lassitude with slight morning cough for two months prior to admission. A half-sister aged eight years was found to be Moro positive at routine examination. On examination of the family at the Central Dispensary for Tuberculosis, a solid infiltration was found in our patient in the left lung. She was Moro negative and remained so during the eight-week

period of hospitalization. No tubercle bacilli were found on culture. The cold agglutinin and ornithosis reactions were negative. At operation in the Department of Thoracic Surgery, Øresundshospitalet, a cyst the size of a walnut was found in the paravertebral sulcus with a thin neurovascular pedicle attached to the medial part of the fourth intercostal space but unconnected with the sympathetic trunk or the spinal cord. Microscopic examination showed fibrillary connective tissue in the wall of the cyst with normal glands and several layers of cylindrical epithelium on the inner surface. The cyst was probably bronchogenic.

#### Meeting May 22, 1960

##### *A. Sell: Embryological Investigations of the Human Alimentary Canal*

A revised conception of the development of the alimentary canal based upon investigations of approximately 500 human foetuses is given. Development takes place in an oral-anal direction. The duodenum develops partly from the most proximal part of the foregut and partly from the most proximal part of the midgut. The part of the duodenum which originates from the foregut, develops very early and leaves the midgut between the postarterial midgut segment and the hindgut, so that the small intestine migrates from the right to the left side of the abdomen. Simultaneously, the intra-umbilical coils of intestine are "lifted" into the abdomen. In contrast to previous conceptions, it was demonstrated that the last part of the small intestine to come into the abdomen from the umbilical hernia is the portion immediately proximal to the terminal coils of ileum (corresponding to the transition between the pre- and postarterial sections of the midgut) rather than the caecum along with the terminal coils of ileum. After migrating into the abdomen, the caecum lies at the level of the umbilicus and is thereafter displaced over to the right side of the abdomen but still remains on a level with the umbilicus on the anterior surface of the inferior pole of the

right kidney. At this site, the rotation (a total of  $270^\circ$ ) of the caeco-ascending colon is completed at the same time as it descends into the right iliac fossa. The colon continues to grow in length, the flexures form and, finally, the mesenteric attachments are established.

Isolated pathological findings encountered in the foetuses examined are mentioned. Several cases of dystopia coeci proximo-lateralis at various stages of development were found. This developmental anomaly is probably due to the fact that the caecum and the terminal coils of intestine are the last to come in from the physiological small intestinal hernia and are prevented from rotating and (therefore?) are abnormally situated. The earlier theory of cessation of development at a stage where the caecum lies up under the liver can no longer be accepted, as the caecum is never found so high at any stage in the normal development. Finally, the conditions in malrotation are mentioned. From embryological investigations compared with radiological findings, it is considered possible to postulate the hypothesis that malrotation (non-rotation) of the intestine may be due to incomplete development of the oral-duodenal segment, so that the normal development, outlined above, does not occur. In cases of malrotation, it has been found that

the bile duct opens abnormally close to the pylorus immediately distal to the bulbus duodeni. Finally, cases of cascade stomach, coeco-ascendens breve, omphalocele and colon elongatum are mentioned.

#### *E. Vad: Preliminary Experience with Air Ionization*

Some examples of conditions caused by atmospheric electricity such as Föhn and mountain sickness are mentioned. The number of ions in the air amounts to approximately 700–800 per cc. The number depends upon the relationship between the rate of destruction and the rate of formation. The ions are formed by radioactive, cosmic and ultraviolet radiation. Certain observations suggest that there is a biologically optimal concentration and an optimal proportion between positive and negative ions. Passive increase of the ion level may be achieved by being certain that the ions formed naturally are given as good conditions of existence as possible. Active increase is achieved by various forms of ion generators. A follow-up investigation of 118 patients with asthmatic respiratory difficulties treated with ion therapy (Floraion) revealed that 62 per cent had improved but only 41 per cent to such an extent that it was of practical therapeutic significance. Forty-five of the patients were under the age of 15 years. In this age group, improvement occurred in 69 per cent and considerable improvement in a total of 52 per cent. Finally, some experiments with patients placed on highly insulated beds are mentioned. It is possible to demonstrate intake of electricity from the air corresponding to a current strength of  $10^{-8}$  to  $10^{-9}$  A, when a potential of 4000–5000 V is applied to the patient. The strength of the current increases if an ion producer is placed in the vicinity of the patient. The increase depends upon the strength of the ion producer and whether the patient faces the ion producer or not.

**DISCUSSION.** *Sv. Heinild:* Many patients report a sense of well-being following radiation with ultraviolet light. Is this due to increase of ionization? — *P. Bechgaard:* Dr

Vad has not drawn any conclusions from the investigations but, after having followed Bach's work with the Floraion apparatus, we must state that we know too little about the significance of the electric conditions for man. We will now attempt to undertake measurements in this respect. It has been demonstrated that some patients are able to become charged with electricity to an unknown extent and they feel uncomfortable. Such individuals should avoid nylon clothes etc. We have been able to cause dyspnoea by alteration of the ionization of the air but our knowledge of the whole subject is in its infancy. — *E. Vad:* The effect of mercury-quartz light may, by and large, be attributed to ionic action. Ion therapy is most effective when the patient is grounded. — *Engineer Bach:* Many individuals may become charged with static electricity so that long sparks form. In a room without green plants, with dry air, plastic paint on the walls, floor and furniture, an individual may become charged with 50,000 to 100,000 volts by walking across the carpet in leather shoes. I have investigated the humidity of such a room and demonstrated that 150 kg of water were necessary to establish the normal humidity. Many people do not feel comfortable in such rooms. Antistatic treatment of the furniture, floor and walls may improve conditions, as this treatment permits normal discharging to take place.

#### *G. Gregersen: A Fifteen-year Material of Meningitis and Follow-up Examination from a Central Hospital*

The material consists of 736 patients admitted to the Central Hospital in Randers during the period 1945–1959. Forty-two per cent of all of the cases were purulent. The majority of the patients were children under the age of 16 years. Follow-up investigation was undertaken by sending questionnaires to the 447 patients who survived during the last ten years. Replies were received from 89.7 per cent. Permanent sequelae were found in 24 per cent of the cases of meningococcal meningitis, 77 per cent of H.influenzae and 54 per cent of pneumococcal meningitis. If

paralyses due to poliomyelitis were excluded, sequelae were found in 29 per cent of the cases of serous meningitis. Serious sequelae were concerned in only a small percentage. The most frequent findings were.

	Purulent %	Serous %
Headache	12	8.4
Vertigo	4.5	7.2
Impaired hearing	9	1.2
Reduced mental capacity or delayed development	10.5	3
Irritability and neurasthenic complaints	12	10

No definite difference in the incidence of the sequelae was found in the various age groups. On the other hand, the incidence of sequelae was increased when the sugar in the

CSF was less than 20 mg % or when pyrexia had been present for more than six days prior to admission.

**DISCUSSION.** *H. Dyggve:* Meningitis frequently begins insidiously in new-born infants and there are relatively many sequelae. Of 20 cases of meningitis in new-born infants diagnosed at the University Hospital in the past ten years, eight died and four developed hydrocephalus. Seven of the patients were premature. The meningitis was due to *B. coli* in eight cases and of these four patients survived but three had hydrocephalus. I would recommend that treatment with streptomycin, sulpha preparations and tetracyclines be commenced as soon as the diagnosis of meningitis is established in a new-born infant rather than wait for a bacteriological report.

*Folke Tudvad, Copenhagen*

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## BOOK REVIEWS

**E. Denhoff and Isabel Robinault: Cerebral Palsy and Related Disorders. A developmental Approach to Dysfunction.**

McGraw-Hill Company London, 1960. Four hundred and twenty-one pages. Price 93 s.

The book contains a comprehensive and thorough review of the rapidly accumulating literature on cerebral palsy, as well as of the authors' own vast experience on the subject. It is stressed that the condition is but one neuromotor aspect of a brain-injured child and cannot be isolated from other cerebral dysfunctions such as mental deficiency, epilepsy, the hyperkinetic-behavior disorder, and the sensory disabilities. The developmental approach to these disorders implies that the manifestations may change as the child develops and these may have prognostic consequences. For example, the hyperkinetic behavior syndrome tends to disappear with maturation. Although the patient's neuromotor handicap frequently persists the dynamic approach which is described by the authors forms the basis for the evaluation and treatment of these children, as practiced in the Meeting Street School in Providence. The associated sensory and perceptual-motor disturbances are dealt with in great detail and this chapter is especially instructive for those regarding cerebral palsy more or less as a simple orthopedic problem. However, no matter how well these cerebral-palsied children are taken care of, the challenge of today is the poor social prognosis even for those with normal intelligence and only a moderate motor handicap. Emotional and psychological factors apparently play an important role in this respect with ensuing difficulties for the young adult in finding a place and an occupation in the community or at least a meaningful existence. Educational and voca-

tional guidance are apparently not sufficient as reflected in the low rate of employment. The monograph can be wholeheartedly recommended as a most useful source of information for all categories working in the cerebral palsy team.

*Bo Hellström, Stockholm*

**Ed. by A. Hottinger and H. Berger: Modern Problems in Pediatrics, Vol. VI. Renal Function and Kidney Disorders in Childhood.**

S. Karger A.G., Basel, 1960. 560 pages. Price S.Fr. 98:—.

The new volume in this series consists of 27 papers dealing with current problems in renal physiology and pathology, with special emphasis on the pediatric age group. Most of the authors are well known authorities in the field. The purpose of the articles is either to review the most recent investigations or to focus attention on a limited aspect of a particular topic. No original communications are presented. It is not feasible to review all papers in this book. If only some are mentioned it is because they have been of special interest to the reviewer. McCrory, discussing glomerulonephritis in childhood, closes his paper with a very concise—and from the clinical point of view, valuable—delineation of the obscure disease chronic nephritis. The overwhelming literature on the nephrotic syndrome is now almost impossible to follow. However, Kretschmer, Barnett & Shibuya present the essentials of recent investigation in this field and stress the modern concept that the nephrotic syndrome is not a disease but a group of symptoms, which may appear in a variety of situations. Linneweh writes briefly and elucidatingly about acute pyelonephritis, especially the oligosymptomatic variety of



this disease. Royer gives a most authoritative description of thrombotic micro-angiopathy or Moschcowitz's syndrome, a disease which is being recognized with increasing frequency. Examples of other diseases given attention from diagnostic, functional and therapeutic points of view are: obstructive uropathy, nephrolithiasis, renal vein thrombosis and acute renal insufficiency. Different aspects of water metabolism and of renal function of the newborn are explicitly treated in four papers. In one of these Wirz gives a short description of his and his collaborators fundamentally new concept of the mechanism of renal water conservation. Other aspects of renal tubular function such as hydrogen ion metabolism, mineral conservation, and mellituria are discussed in several papers.

The standard of the papers is somewhat uneven—a more concise presentation of the content of some articles would have been desirable—but on the whole this book provides informative reading, not only for those who have not had the opportunity to follow the advances in renal pediatrics, but also for those with special interests in this this field.

*Jan Winberg, Gothenburg*

**J. W. Miller, S. D. M. Court, W. S. Watson and E. G. Knox.** *Growing Up in Newcastle Upon Tyne.*

Published for the Nuffield Foundation by Oxford University Press, London. 1960. Price 25/-net.

In 1941 Sir James Spence and his collaborators at the Children's Department of Kings College and the Health Department of Newcastle began an enterprise that is well known all over the world: "The 1000 Family Survey". The primary object was to obtain an accurate record of the incidence and types of infectious illnesses in infancy. During the survey the study was enlarged to comprise the lives and health of children. The first volume with a report of the childrens' first year of life was published in 1954 and was reviewed in *Acta Paediatrica* 1955.

After the death of Sir James his resident Dr. Miller with co-workers has followed-up the survey and they have now published this second volume which concerns the first five years of the childrens' lives. The number of children involved was 847. Only 7 families had withdrawn from the survey. During the 5-year period an additional 507 children have been born within the original 1000 families. In comparison with the previous study a greater feeling and regard for the welfare of children and concern for their health was noted, but only too often one or more of the essentials required to establish a stable and happy home were lacking. The book is a real goldmine of information for the study of young children in health and sickness in a community and contains an almost overwhelming amount of data. Only a small part of this can be quoted.

Infectious illness caused 80 % of the morbidity in the first 5 years and formed the bulk of the family doctor's work with children. Respiratory infections accounted for more than half of the total infections. An outbreak of diphtheria in 14 children, of whom 2 died, was traced to a visit to Vienna by one of the families. Eight of the affected children had been adequately immunized. The authors describe what they call "staphylococcal families" with an abnormal susceptibility to recurrent staphylococcal infections. Their incidence is estimated as 8-10 in 3000 patients. Sixty-one of the 847 children were infected with tuberculosis (=7.2 %), 11 during the first year of life. Five of the 23 children infected before the age of 2 years developed meningitis of whom 4 died and the 5th became mentally retarded. We do not get any information about the percentage of BCG vaccinated children. Tuberculosis has declined very much in Newcastle and the authors stress that not more than 2 % of school entrants were tuberculin positive in 1957. The number of episodes of non-infectious illnesses was 1322. There were 732 accidents recorded in the first 5 years, almost one accident per child and these constituted 8.5 % of the total illnesses. A little more than half of the ac-

idents occurred at home. There were 3 deaths from accidents, all in the first year, and 4 children have persisting disabilities. Prevention of accidents on a community basis is discussed. The number of children with congenital defects has increased to 23 from 18 discovered during the first year of life, corresponding to 2 % of all newborn children. Forty-nine children had had one or more fits before going to school. No less than 383 of the 847 children suffered from deprivation of parental care. In 21.6 % of the families there were deficiencies of care regarding diet, clothing and cleanliness and 20.2 % of the families had some form of social aid. There were 67 illegitimate children of which 7 had died in the first year of life. Only 11 children did not receive the attention of a doctor during these 5 years. The attendance in the Child Welfare Centres (at least once) decreased from 67 % during the first year to 10 % during the fifth and the figures for attendance at least 6 times a year fell from 31 % to 2 %.

The book ends with 3 pages of conclusions and suggestions. The authors state how they think the care of children could be further improved and what type of help parents need. They especially stress the need of more well-constructed dwellings and they are concerned that so many mothers with young children are compelled to work outside their home.

***Antibiotics in Medicine.* British Medical Bulletin.**

Vol. 16, Nr 1 Jan. 1960. 65 Davies Street, London, W. 1. England. Price £ 1.

Since the pediatrician deals with the use of antibiotics in his daily work in hospital and practice he cannot really afford to be ignorant of or even behind in this matter. The British Medical Bulletin has a whole issue "Antibiotics in Medicine" to offer. The articles by outstanding English authors are very valuable. The main interest is focused upon the general aspects of antibiotic therapy. A comprehensive account of therapeutic uses is out of question because of the

limitations of space. Nevertheless, four contributions are concerned with specific clinical uses; preventative administration in medicine and surgery, treatment of tuberculosis and bacterial endocarditis. The remaining contributions deal with more general matters, like the chemical nature and the relationships of antibiotics, the methods of their employment, their mechanism of effect, their beneficial and ill effects, acquired bacterial resistance to antibiotics, determination of bacterial sensitivity and the use of antibiotics in selective culture media. The many profound problems of antibiotic therapy, such as the questions of interaction between the individual cell and the antibiotic, the general necessity for combined treatment and the extent of preventative use of antibiotics, cannot be settled in this symposium. The attempt is to provide the reader with well-documented information on which he can base his own opinion, and with information which is not obtainable in books of the very rapid and complex developments in this particular field of medicine.

*Ole Wasz-Höckert, Helsingfors*

**Advances in Pediatrics Volume XI, 1960. Z. Levine (Ed.). The Year Book Publishers, Chicago, Ill.**

The eleventh volume of *Advances in Pediatrics* offers its reader six different topics: 1, respiratory problems in the newborn period; 2, interrelations between sodium metabolism and brain function; 3, allergy in childhood; 4, treatment of enteric dehydration; 5, aminoaciduria and aminoacid metabolism; 6, steroid treatment in rheumatic carditis. The chapters are well written by qualified people. The references are complete and up-to-date.

Monographs 1, 2, and 5 are primarily review articles; here the voluminous, often recently obtained knowledge has been summarized in paragraphs which are easy to read and understand. Monographs 3, 4 and 6 emphasize the practical therapeutics of these common pediatric problems and should be a

useful guide to the clinician, particularly since each is written by an author with long-standing experience in his subject.

Like other volumes of *Advances in Pediatrics*, the eleventh is written so that it can also be understood by the physician who is not too familiar with the particular field at hand.

*G. Wallgren, Stockholm*

**Franz Schmid und Helmut Moll: Atlas der normalen und pathologischen Handskelettentwicklung.**

Springer Verlag, Berlin, Göttingen, Heidelberg, 1960. Price; 78 DM.

10 tables, 113 fig., 114 pp.

This book is composed of two parts: an atlas and a clinical-diagnostic study. The atlas, which contains no less than 70 reproductions, forms the basis for the estimation of normal and abnormal values for the rate of appearance, size and configuration of the skeletal elements of the hand. It is richly supplemented by tables, which present the normal variations in size of the carpal bones, as well as height and weight of normal subjects from the neonatal period up to 14 years of age. Additional tables give a condensed survey of the appearance time for the carpal bones and the epiphyseal centers of the hand, and the relation between the height of the individual and the length of the hand. In two tables the length of the humerus, radius and ulna is related to age and height of the normal individual. The relationship

between the size of the carpal bones and height of the body is also given.

In the second part, an extensive account is presented of the diagnostic significance of abnormalities in the different bones of the hand as to number, order and rate of appearance, configuration and disturbances of mineralization. This section is also richly illustrated. The reproductions are, without exception, of high quality. In a particularly interesting chapter, the reflection of disturbances in the central nervous system on the skeletal development of the hand is briefly described. Deviations from normal are common and some of them have diagnostic significance. It follows that such patients are not feasible subjects for studies of normal variations in skeletal development, a point on which enough emphasis has not invariably been placed in earlier treatises.

According to the present study, radiographic examination of the hand with due consideration to its length, the size of its bony elements and their rate and order of appearance, should obviate the need for more extensive radiologic examinations of the extremities for determining skeletal age in daily practice. It is obvious that this means, among other things, a clear reduction in radiation dose.

In the reviewer's opinion, this book is an outstanding contribution to a subject that interests paediatricians and radiologists alike. It can be warmly recommended.

*Ulf Rudhe, Stockholm*

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## ANNOUNCEMENT

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### The X International Congress of Pediatrics

The X International Congress of Pediatrics will be held in Lisbon, Portugal Sept. 9 to 15, 1962 with Professor C. Salazar de Sousa as President. An excellent scientific program is being arranged with translation services for French and English. An exten-

sive social program is planned as are scientific exhibits, industrial exhibits, and exhibitions of art and children's literature. The address of the Secretariat: Av. 28 de Maio, Lisbon, Portugal.

FROM the Public Health Laboratory, Manchester, and the Streptococcus and Staphylococcus Reference Laboratory, Colindale, London

## Further Observations on the Bacteriology of Impetigo and Pemphigus Neonatorum

by M. T. PARKER and R. E. O. WILLIAMS

The two organisms most often considered as possible causes of *impetigo contagiosa* are *Staphylococcus aureus* and *Streptococcus pyogenes*. They have been isolated in widely varying proportions from series of cases examined at different times and in different parts of the world. The idea that there are two clinical forms of the disease, one staphylococcal and the other streptococcal, was first put forward clearly by Engman (1901). He considered that the classical *impetigo contagiosa* of Dunn (1863) and Fox (1864), which is characterised by a relatively thick "stuck-on" crust, was a streptococcal disease, and described in addition a bullous type from which *Staph. aureus* could be isolated, usually in pure culture. The close resemblance between the bullous type of impetigo and *pemphigus neonatorum* was also noted many years ago, and several workers considered that they were essentially the same disease, due to strains of *Staph. aureus* with a special ability to give rise to an intraepidermal vesicular lesion (1, 5, 15), but numerous attempts to show that they differed culturally from *Staph. au-*

*reus* strains from boils and abscesses were unsuccessful (9).

In 1955, Parker, Tomlinson & Williams described an epidemic of impetigo in Salford, Lancashire, in a which *Staph. aureus* was isolated from 83 % and *Str. pyogenes* from 47 % of the lesions. They found that three-quarters of the staphylococci belonged to a single phage type (Type 71) which was not commonly found in other sorts of lesion and was rare in deep suppurations. Also, 78 % of the strains of *Str. pyogenes* gave one of two patterns, 3/13/B3264 and 5/11/12/27/44, when typed by agglutination. In the same year Barrow (3) examined a series of cases of impetigo in Bradford and found that 63 % of the *Staph. aureus* strains belonged to Type 71. He obtained *Str. pyogenes* from only 17 % of his cases, and only one-third of them had the typing patterns commonly observed in Salford. His cases had been seen in a hospital outpatient department, whereas the Salford cases had been swabbed in school minor-ailment clinics. Spittlehouse (22) examined swabs from a series of hospital out-

patients with impetigo in Sheffield during the following year, and also noticed a predominance of *Staph. aureus* Type 71, but he gave no information about the isolation of streptococci.

Although *Staph. aureus* Type 71 did not predominate in any lesion other than impetigo, the Manchester workers noticed that it was found fairly often in bullous and pustular skin infections of newborn infants, and in 1957 Gillespie, Pope & Simpson described a hospital outbreak of *pemphigus neonatorum* due to it.

Type 71 staphylococci have a number of unusual cultural characters. They inhibit the growth of *Corynebacterium diphtheriae* in surface cultures on blood agar with the production of a characteristic sharply-demarcated zone, and they produce opacity on serum agar but not in an egg yolk medium (17). The use of these tests has made it possible to recognise a small number of other strains with '71-like' characters. They, too, are found exclusively in superficial situations, and are associated with impetigo and *pemphigus neonatorum*.

It is tempting to believe that *Staph. aureus* Type 71 and *Str. pyogenes* 'types' 3/13/B3264 and 5/11/12/27/44 are the causes respectively of staphylococcal and streptococcal impetigo. The significance of the very common mixed infections, in which both *Staph. aureus* and *Str. pyogenes* are found in the same lesion, has still to be explained. In the present paper we summarise information obtained on the bacteriology of impetigo in south-east Lancashire in the years 1953-7 and compare the frequency of occurrence of certain staphylococcal and streptococcal types, alone and in combination, in cases seen in school clinics and in a hospital out-patient de-

partment. We also give further information about the association of *Staph. aureus* Type 71 with infections of newborn infants, including *pemphigus neonatorum*.

## Materials and Methods

Between September 1953 and August 1957 swabs from 514 cases of impetigo in children were received, nearly all from school clinics, 403 of them in Salford and the rest in neighbouring towns. Also in the years 1955-7, 95 patients diagnosed as impetigo in the Out-Patient Department of the Manchester & Salford Skin Hospital by Dr. J. K. Morgan and Dr. K. R. Haye were swabbed when they first attended at the hospital. All swabs were cultured on blood agar and on gentian violet blood agar, and strains of staphylococci and streptococci were isolated and identified by methods described previously (19). During the 4 years from September 1953, 532 strains of *Staph. aureus* from lesions of newborn infants, mainly but not exclusively infected in hospital, were collected.

Strains of *Str. pyogenes* were typed both by the slide agglutination (14) and by the precipitation (23) methods. Very few of the streptococci were typable by the M precipitin test and many gave complex agglutination patterns with T sera for several types. A strain described as having, for example, the agglutination pattern 3/13/B3264 was one which was agglutinated by two or more of these T sera but gave no M precipitation reaction.

Strains of *Staph. aureus* were phage-typed as described by Anderson & Williams (2), using the basic set of phages recommended by the International Committee on Phage Typing (Report, 1959), except for phage 80, which was only included after August 1955. They were also subjected to the following tests: penicillin resistance, inhibitory action on *C. diphtheriae*, the egg yolk reaction, and the opacity reaction on horse serum agar, as described previously (7).

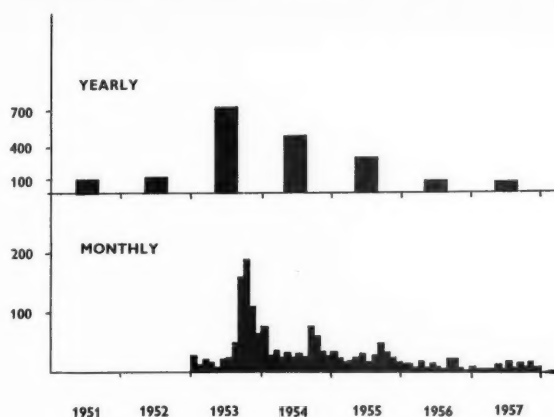


Fig. 1. Number of cases of impetigo treated in school clinics in Salford.

### Results

Impetigo was found to be unusually prevalent among school-children in Salford when the schools re-assembled after the summer holidays in 1953. Many of the cases were exceptionally severe, and were characterised by the presence of large bullae. Lesions occurred not only on the face and neck, but also on the chest, abdomen, buttocks, hands and arms (4). At about the same time it was reported from the neighbouring borough of Eccles that an unusual bullous skin infection had

become common among school-children. The information in Fig. 1, which shows the annual and monthly numbers of cases of impetigo treated in Salford Health Department clinics, was provided by Dr. J. L. Burn. In his opinion, the figures for the peak of the 1953 outbreak are somewhat inflated by an intensive search for cases made in October and November. The outbreak was probably already under way when the school term began in September, and continued through the autumn and the early part of the winter. There was a con-

TABLE 1. *Presence of Staphylococcus aureus and of haemolytic streptococci in lesion swabs of cases of impetigo.*

Number of lesions	School clinics			Skin Hospital 1955-57
	1953-54	1954-55	1955-57	
Total	190	183	141	95
containing <i>Staph. aureus</i>	158 (83%)	155 (85%)	114 (81%)	92 (97%)
<i>Str. pyogenes</i>	90 (47%)	91 (50%)	63 (45%)	12 (12%)
Haemolytic streptococci Group C	8	2	4	3
Haemolytic streptococci Group G	2	2	1	0
No <i>Staph. aureus</i> or haemolytic streptococci	6	11	10	2



TABLE 2. *Phage-typing of Staphylococcus aureus strains from impetigo lesions*

Phage group	School clinics			Skin Hospital	Total
	1953-54	1954-55	1955-57	1955-57	
I.	4	4	16	6	30
II. { Type 71	119 (75 %)	84 (54 %)	54 (47 %)	69 (75 %)	326 (63 %)
{ "71-like"	2	6	3	3	14
{ Other patterns	7	13	9	1	30
III.	8	15	10	8	41
Not typable { "71-like"	1	12	2	0	15
{ Other	12	18	10	3	43
Unclassifiable	5	3	10	2	20
Total Type 71 & "71-like" strains	122 (78 %)	102 (66 %)	59 (52 %)	72 (78 %)	355 (68 %)
Total	158	155	114	92	519

siderable falling-off of cases in February 1954. Small autumnal peaks occurred in 1954 and 1955, but thereafter the incidence was low and non-seasonal.

#### *Bacteriology of Impetigo Lesions*

The results of the bacteriological examination of one lesion from each of 609 patients are summarised in Table 1. It was convenient to divide the material from the school-clinics according to the school year, which begins in September, and since the number of cases examined in the two years 1955-56 and 1956-57 was rather small they will be considered together. About 80 per cent of the swabs examined in each period were from cases in Salford, where a systematic study of the disease was being made. No difference could be found between the results of the Salford swabs and those from neighbouring towns, and they will not be considered separately. As might have been expected, the proportion of notified cases examined bacteriologically was lowest when the disease was most common. Swabs were received from

20 % of the recorded cases in 1953-54, but the proportion had risen to 70 % by 1956-57. Despite the fall in the incidence of the disease during the period of the investigation, there was little change in the proportion of swabs yielding *Staph. aureus* and *Str. pyogenes*. In each year four-fifths of all lesions contained *Staph. aureus*. Just under half contained only *Staph. aureus*, one-third yielded both *Staph. aureus* and *Str. pyogenes* and a little over one-tenth *Str. pyogenes* alone. The findings in the series of hospital out-patients, examined during the 2 years in which the disease appeared to be relatively uncommon, and shown in the last column of Table 1, were entirely different. Here, 97 % of the lesions contained *Staph. aureus*, but *Str. pyogenes* was present in only 12 %. The results were, in fact, very like those obtained by Barrow (3), from hospital out-patients, in Bradford in 1953-54.

Table 2 gives the results of phage-typing the *Staph. aureus* strains and shows the proportion belonging to Type 71 and to certain other groups. Type 71 was by far



TABLE 3. *Presence of Streptococcus pyogenes strains reacting with sera 3/13/B3264, 5/11/12/27/44 & 8/25/Imp. 19 in impetigo lesions.*

Strains described as "mixed" reacted with 2 or more sera of each of the two groups 3/13/B3264 & 5/11/12/27/44).

	School clinics			Skin Hospital 1955-57	Total
	1953-54	1954-55	1955-57		
Total <i>Str. pyogenes</i>	90 <sup>a</sup>	91	63	12	256
3/13/B3264	33	35	32	3	103
5/11/12/27/44	34	36	11	3	84
"Mixed"	0	3	0	1	4
8/25/Imp. 19	2	2	12	0	16
Other types	17	15	8	5	45

<sup>a</sup> 4 strains not typed.

the most commonly encountered type in all years, and the rest of the strains included a great variety of different phage patterns, none of which occurred frequently. There was a steady fall in the proportion of Type 71 strains in the school-clinic series from 75 % in 1953-54 to 47 % in 1955-57. In the hospital out-patient series, the percentage in 1955-57 was as high as it had been in the school clinics at the peak of the impetigo epidemic. Table 2 also shows that a number of strains in the rest of phage Group II, and of non-typable strains, with '71-like' characters were also isolated: these strains all gave 'sharp' inhibition-zones on *C. diphtheriae* and were egg-yolk negative. If they are accepted as possible causes of impetigo, the difference between the findings in 1953-54 and 1954-55 would be somewhat reduced.

In Table 3 the results of typing strains of *Str. pyogenes* are shown. In both 1953-54 and 1954-55, the two impetigo 'types' 3/13/B3264 and 5/11/12/27/44 together made up 70 % of all strains, but in the last 2 years there was a relative deficiency of 5/11/12/27/44 strains. It is inter-

esting to note that during this latter period a number of strains agglutinated by the sera 8/25/Imp 19 were isolated, as similar strains had made up nearly one-third of a series from impetigo among troops examined by Dr. N. Crowley in 1941 (see Parker *et al.* (19). Four strains reacting with 2 sera from each of the groups 3/13/B3264 and 5/11/12/27/44 (recorded as 'mixed' strains in Table 3) were also seen.

Some further indication of the importance of the various types can be obtained from the streptococcus type distribution surveys carried out twice a year by the Public Health Laboratory Service (Report 1957 and further unpublished results). In 13 surveys there were streptococci from a total of 60 cases of impetigo: 35 % had agglutination patterns in 3/13/B3264, 17 % in 5/11/12/27/44, and 5 % in 8/25. The percentage among the 3660 streptococci from all sources were, respectively, 5, 12, and 6. The excess of strains in the 3/13/B3264 group is therefore clear; that in the 5/11/12/27/44 group is less clear; and there is no excess in the 8/25 group.

The serological reactions of the impetigo

TABLE 4. *The association of specific types of Staphylococcus aureus and of Streptococcus pyogenes in patients with impetigo.*

	Number of cases with streptococci			Total
	"types" 3/13/B3264 5/11/12/27/44 or mixed	other types	absent	
Cases with staphylococci				
Type 71 & "71-like;"	65	22	266	353
Other types	76	30	57	163
Absent	50	9	30	89
Total	191	61	353	605

streptococci have also been investigated in more detail. Using the methods for absorption of typing sera described by McLean (16), it has been found that most recently isolated impetigo strains in the agglutination group 5/11/12/27/44 are probably in fact Type 11. There is some indication that impetigo streptococci, whether in the 3/13/B3264 or the 5/11/12/27/44 group, may share a common antigen not found in strains from other lesions (Fraser, unpublished observations). These relationships will require further study, but they may well lend support to the idea that there are specific characters associated with the streptococci that cause impetigo.

#### *The Association between Staphylococcal and Streptococcal Types in Impetigo Lesions*

If impetigo may be either staphylococcal or streptococcal, and only certain strains of either organism can cause it, one might expect that the staphylococcal cases would yield the specific staphylococcus but that the streptococci found in them might be of any type, and conversely that the streptococcal cases might have a variety of types of staphylococci.

For Table 4 the specific impetigo staphylococci were taken as those of Type 71 and the '71-like' Group II and untypable

strains; impetigo streptococci were those with agglutination patterns 3/13/B3264 or 5/11/12/27/44 or mixtures of the two.

Of the 353 cases yielding the specific staphylococci, 87 (25%) yielded some streptococci whereas of the 252 cases not yielding impetigo staphylococci, 165 (66%) yielded streptococci. In both groups, however, some 76% of the streptococci belonged to the impetigo types. In the P.H.L.S. surveys, 17% of all streptococci belonged to these types.

There were 191 cases with impetigo streptococci and 141 (74%) of these yielded staphylococci; there were 414 cases with no impetigo streptococci and 375 (91%) yielded staphylococci. In the two groups the proportion of the staphylococci in the impetigo types were 46% and 77% respectively. In a survey of staphylococci in lesions other than impetigo in out-patients (17), only 7% belonged to Type 71. Williams (25) found only 2% of Type 71 strains among staphylococci from septic lesions in hospital patients.

It seems, then, that when 'impetigo' staphylococci were absent there was an increased frequency of streptococci, and conversely. But whether staphylococci were present or not, about 76% of the

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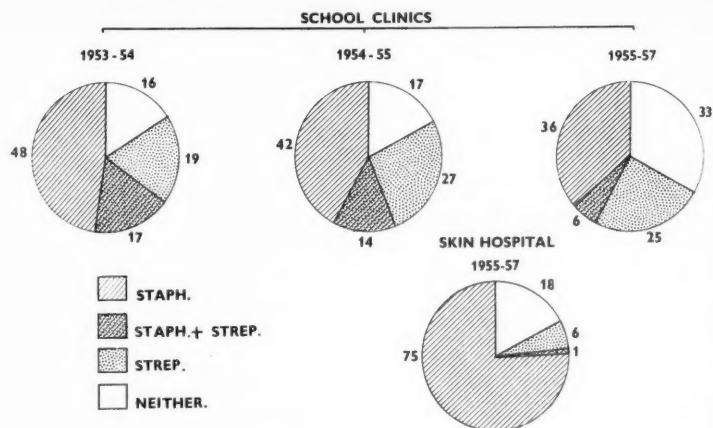


Fig. 2. Percentage of lesions from which impetigo streptococci and impetigo staphylococci were isolated alone or together.

streptococci were of the specific impetigo types, as though perhaps these types were especially well adapted to life on the skin. In all cases a large proportion of the staphylococci belonged to Type 71 but the proportion was greater in lesions yielding no streptococci.

Fig. 2 shows the percentage of lesions associated with impetigo staphylococci and impetigo streptococci alone or together. If impetigo is considered staphylococcal when it contains only *Staph. aureus* Type 71 or a '71-like' strain, streptococcal when it contains only *Str. pyogenes* with agglutination patterns in 3/13/B3264, 5/11/12/27/44 or in both, and mixed when it contains both staphylococci and streptococci of impetigo types, then 43 % of the school clinic cases were presumably staphylococcal 24 % streptococcal and 12 % mixed. The percentage of staphylococcal cases fell steadily as impetigo became less common (48 % in 1953-54: 36 % in 1955-57). Most of the mixed cases occurred in the early years, and streptococcal cases were com-

moner in the later years than in 1953-54. The percentage of cases from which no specific organism was isolated was greatest when the incidence of the disease was lowest. In the series of hospital outpatients, 75 % of the cases were presumably staphylococcal, 6 % streptococcal and 1 % mixed.

#### *Staphylococcal Infections of Newborn Infants*

Between September 1953 and August 1957, 532 strains of *Staph. aureus* from infant lesions were received for typing at the Manchester Public Health Laboratory. They included representative strains from 34 outbreaks of sepsis and 13 sporadic infections in 23 hospitals in north-west England. No attempt was made to select the material, which represents those incidents in which hospital pathologists considered that they needed the help of phage-typing to investigate and control an outbreak. Staphylococci were also obtained from 1 small outbreak and 3 sporadic infections in infants born at home.

Table 5 summarises the typing results on the strains from all sorts of lesions. As

TABLE 5. *Phage-typing of 532 Staphylococcus aureus strains from lesions of newborn infants.*

Phage group	Skin: vesicles, bullae or spots	Other skin lesions	Umbilical sepsis	Conjunc- tivitis	Deep suppuration
I.	92	3	39	49	28
II. { Type 71	67	2	0	7	1
{ "71-like" A	3	0	0	1	0
{ "71-like" B	9	1	0	2	0
{ Other patterns	7	0	1	4	2
III.	43	4	31	35	9
Not typable { "71-like"	1	0	0	0	0
{ Other	39	2	8	25	4
Unclassifiable	2	0	5	6	0
Total	263	12	84	129	44

is usual, representatives of a great many phage patterns were found. Only 14 % of all the strains, but 25 % of those from vesicles, bullae and 'spots' on the skin belonged to Type 71. Only 6 % of strains from other superficial situations, and 2 % from deep lesions were of this type. Phage Group II strains other than Type 71 were uncommon, as is usual in hospitals. It is interesting to note that over half of them were not Group II strains of the sort commonly found in boils and abscesses in general practice, but were '71-like'. Those referred to as '71-like A' were identical with the strains seen in impetigo lesions, and those described as '71-like B' all had the phage-pattern 55/71, did not inhibit *C. diphtheriae* but were egg-yolk negative and penicillin resistant. They were therefore identical with the 'DI negative' variant which appears in practically all Type 71 cultures on storage in the laboratory (18). Such strains were also isolated in some outbreaks in close epidemiological association with ordinary Type 71 strains, and in one outbreak both forms were isolated from each of two patients on the same occasion.

In Table 6, information is given about the 35 outbreaks and 17 sporadic cases from which these strains had been isolated. It shows the phage-typing results on all strains responsible for skin sepsis in each incident; strains obtained only from 'sticky eye' or from trivial umbilical infections are excluded. In 26 outbreaks only one strain was involved, but 24 different strains were isolated in the remaining 9 outbreaks. Although Type 71 was responsible for only a quarter of all the skin spots, it was one of the infecting types in 19/35 (54 %) of all outbreaks, and was the only strain isolated in 14/26 (54 %) of outbreaks due to one type. Two '71-like' strains also gave rise to outbreaks. The average size of the outbreaks due to Type 71 was rather small (usually 5 cases or less). In the last column of Table 6 are the 17 strains from single cases of skin sepsis, 14 arising in hospital and 3 in babies born at home. Ten were due to Type 71, including all three of the home deliveries. One of the latter appeared to have been contracted from an older child with impetigo.

We obtained accounts of the clinical

TABLE 6. *Phage-typing of the strains of Staphylococcus aureus responsible for 35 outbreaks & 17 sporadic cases of skin sepsis in newborn infants.*

Phage group	All strains	Outbreaks due to a single strain	Sporadic cases
I.	14	5	0
II. { Type 71	19 <sup>a</sup>	14 <sup>b</sup>	10
{ "71-like" A	1	1	0
{ "71-like" B	1	1	0
{ Other patterns	0	0	1
III.	12	4	0
Not typable { "71-like"	0	0	1
{ Other	3	1	5
Unclassifiable	0	0	0
Total	50	26	17

<sup>a</sup> 3 with associated cases due to "71-like" B.<sup>b</sup> 2 with associated cases due to "71-like" B.

condition of many of the infants who had skin infections due to *Staph. aureus* Type 71. The lesions varied greatly in severity, but the characteristic appearance was of a superficial blister with little surrounding inflammatory reaction. Sometimes only a few small vesicles were present, at others large, confluent bullae. Occasionally a generalised exfoliative dermatitis occurred. We have encountered four such cases which were diagnosed clinically as *Von Ritter's disease*. Two of these formed part of a small outbreak consisting of two cases of generalised exfoliative dermatitis and one case of impetigo associated with a midwife in domiciliary practice. The first infant died rapidly from an exfoliative dermatitis, from which *Staph. aureus* was grown in pure culture but was not typed. A few days later, a few clear blisters 'like scalds' appeared on the arm of an older sister of the baby, but no cultures were made. The second baby, in a different household in the same village, was delivered by the same midwife about a fortnight later. It suffered from a severe

attack of exfoliative dermatitis but survived. *Staph. aureus* Type 71 was isolated from the skin lesion of the second baby and from the nose of the midwife.

### Discussion

The information presented here lends some support to the view that the superficial vesicular or crusted non-scarring lesions diagnosed clinically as *impetigo contagiosa* include a staphylococcal disease due to *Staph. aureus* Type 71 and a streptococcal disease associated with *Str. pyogenes* 'type' 3/13/B3264 or 5/11/12/27/44.

The material examined consisted of single swabs from the lesions of impetigo cases, seen by a number of medical officers, on first attendance at a school clinic or a hospital out-patient department, and included swabs collected at all stages of the disease. One or more of the organisms thought to be associated with impetigo was isolated from 79 % of the cases. The remaining 21 % may have included some in which the causative organism had dis-

appeared before the lesion was swabbed, some in which its presence was masked by secondary invasion by another strain of the same species, others in which the diagnosis was incorrect, and possibly also cases due to other strains of *Staph. aureus*, or of *Str. pyogenes*, the association of which with impetigo has not yet been recognised.

In the whole series, staphylococcal impetigo appeared to be about twice as common as streptococcal, and there were considerable differences in the relative proportions from year to year. A most unexpected finding, however, was the great difference between the flora of lesions swabbed in school clinics and the out-patient department of the skin hospital. Although staphylococcal cases were at all times more common than streptococcal cases in the school clinics, the difference was not great, except during the impetigo epidemic in 1953, and the two specific types of *Str. pyogenes* were at no time isolated from less than 30 % of the lesions. In the hospital out-patient series, which was examined at a time of low incidence of impetigo in the general population, *Staph. aureus* Type 71 was isolated from nearly three-quarters of the cases, and *Str. pyogenes* was seldom seen. A number of possible explanations of this were considered. It might be thought that *Str. pyogenes* was present frequently in early, untreated cases seen in the school clinics, and that secondary infection with *Staph. aureus*, either naturally or in consequence of penicillin treatment, had caused the disappearance of the streptococci before the cases were referred to the skin hospital. This did not seem to be so, since there was no difference in the frequency with which

*Staph. aureus* Type 71 was isolated from those skin hospital cases seen within 7 days of onset and from those seen later. The possibility that the cases which yielded *Str. pyogenes* were given some other diagnostic label by the physicians at the skin hospital was also considered. A sample of other superficial lesions seen in the same out-patient clinics was examined, but very few strains of the specific 'types' of *Str. pyogenes* were isolated. We were therefore forced to the conclusion that the cases which commonly yielded *Str. pyogenes* did not often reach the hospital out-patient department. This further strengthened our conviction that the school clinic cases included two separate diseases, one of which was not severe enough to be referred to hospital.

Opinion is divided among dermatologists as to whether a clinical distinction between staphylococcal and streptococcal impetigo can be made. It is notable that those who have made the distinction with most confidence have had experience of severe outbreaks of bullous impetigo in which only *Staph. aureus* can be isolated from most of the lesions (6, 8, 10, 24). Such conditions appear to occur frequently in countries with a hot dry summer (8), but the careful clinical observations of Friedberg (12) suggest that the distinction can be made on sporadic cases occurring in temperate climates. It is possible that the clinical diagnosis of staphylococcal impetigo is easily made only when the lesions are severe enough for cases to be seen while the vesicles or bullae are unruptured, and this seems to have been the case during the earlier part of the Oxford epidemic in 1953. At other times the initial vesicle appears often to have been



small, and to have ruptured early. By the time the cases were seen, either in the school clinics or at the skin hospital, the lesion had become crusted and was not very characteristic.

Our findings go some way towards explaining the nature of the mixed infections with *Staph. aureus* and *Str. pyogenes* which made up about one-third of the school clinic cases. The typing results suggested that most of them were secondary staphylococcal infections of streptococcal lesions. However, the relative frequency with which *Staph. aureus* Type 71 and a specific streptococcus were found together in the same lesion remains unexplained.

The part played by *Staph. aureus* Type 71 in the skin infections of newborn infants has been obscured by the fact that it seldom gives rise to large epidemics in hospital, possibly because it seldom becomes widely disseminated among the contacts of cases. In the years 1953-57 it gave rise frequently to single cases and small epidemics in north-west England. It appears to have been rarer in the south of England than in the north during these years, but to have given rise to epidemics of the same general sort in both areas. Williams (25) found only 20 Type 71 strains among 493 'independent' strains from maternity hospitals in southern England in the years 1954-57, and the average number of cases in seven outbreaks was 4.3.

The lesions from which it is isolated consist characteristically of an intraepidermal vesicle with little inflammatory reaction in the deeper tissues, in striking contrast to the more boil-like lesion, often with underlying cellulitis, caused by other strains, including Type 80. Severe lesions

due to Type 71 sometimes occur, and involve large areas of skin, but the lesion is confined to the epidermis.

### Summary

Six hundred and nine impetigo lesions were examined bacteriologically and the presence in them of certain types of *Staph. aureus* and of *Str. pyogenes* thought to be specifically associated with impetigo was determined.

On the basis of the bacteriological findings, 47 % of the cases were considered to be staphylococcal, 21 % streptococcal and 11 % mixed. No specific pathogen was isolated from 21 %.

Among children seen in school minor ailment clinics, staphylococcal cases predominated when impetigo was prevalent, but at all times a fifth or more of the cases were streptococcal. Cases referred to the out-patient department of a skin hospital were, however, nearly all staphylococcal.

It is probable that impetigo includes a more severe, often bullous, skin infection due to *Staph. aureus* Type 71, and a less severe condition associated with certain types of *Str. pyogenes*.

*Staph. aureus* Type 71 was also responsible for a number of outbreaks of skin infection among newborn infants in hospital. Cases were of all degrees of severity from a mild vesicular eruption to a fully developed *pemphigus neonatorum*, and a few cases of generalised exfoliative dermatitis were seen.

Similar cases due to Type 71 were occasionally seen in domiciliary practice, infected either from the midwife or from an older child with staphylococcal impetigo.



The characteristic skin lesion caused by *Staph. aureus* Type 71 is an intraepidermal vesicle or bulla with little inflammatory reaction in the deeper tissues.

### Acknowledgments

We wish to thank the physicians who collected specimens for us and provided clinical

information, and especially Dr. J. L. Burn and Dr. D. E. Jeremiah, of the Salford Public Health Department, Drs. K. R. Haye, C. W. Marsden and J. K. Morgan, of the Manchester & Salford Hospital for Skin Diseases, and Dr. R. W. Kennon. We are also indebted to Miss Jean Perry for preparing the figures.

### References

1. ALMQUIST, E.: Pemphigus neonatorum, bakteriologisch und epidemiologisch beleuchtet. *Z Hyg Infektr*, 10: 253, 1891.
2. ANDERSON, E. S. and WILLIAMS, R. E. O.: Bacteriophage typing of enteric pathogens and staphylococci and its use in epidemiology. *J Clin Path*, 9: 94, 1956.
3. BARROW, G. I.: Clinical and bacteriological aspects of impetigo contagiosa. *J Hyg (Lond)*, 53: 495, 1955.
4. BURN, J. L.: In *Ann Rep M.O.H. City of Salford*, 1953, 1954.
5. CLEGG, M. T. and WHERRY, W. B.: The etiology of pemphigus contagiosus in the tropics. *J Infect Dis*, 3: 165, 1906.
6. DOHL, K. and DOHL, S.: Zur Klinik und Ätiologie der Impetigo contagiosa. *Arch Derm Syph (Wien)*, 111: 629, 1912.
7. DUNN, R. W.: On porrigo. *Brit Med J*, II: 576, 1863.
8. ENGMAN, M. F.: Impetigo contagiosa bullosa and its bacteriology. *J Cutan Dis*, 19: 180, 1901.
9. EPSTEIN, S.: Beitrag zur staphylogenen Impetigo contagiosa. *Derm Z*, 70: 328, 1935.
10. — Staphylococcie impetigo contagiosa. *Arch Derm Syph (Chicago)*, 42: 840, 1940.
11. FOX, W. T.: On impetigo contagiosa, or porrigo. *Brit Med J*, I: 467, 1864.
12. FRIEDBERG, R.: Bacteriological studies on impetigo, especially the streptogenic form. *Acta Dermovener (Stockh)*, 23: 297, 1942.
13. GILLESPIE, W. A., POPE, ROSEMARY, C. and SIMPSON, K.: Pemphigus neonatorum caused by *Staphylococcus aureus*, Type 71. *Brit Med J*, I: 1044, 1957.
14. GRIFFITH, F.: The serological classification of *Streptococcus pyogenes*. *J Hyg (Lond)*, 34: 542, 1934.
15. MATZENAUER, R.: Zur Frage der Identität des Pemphigus neonatorum und der Impetigo contagiosa. *Wien Klin Wochr*, 13: 1077, 1900.
16. McLEAN, SIBELY, J.: Identification of strains of *Streptococcus pyogenes* of types, 5, 11, 12, 27 and 44 by the precipitin test for the T antigen. *J Gen Microbiol*, 9: 110, 1953.
17. PARKER, M. T.: Some cultural characteristics of *Staphylococcus aureus* strains from superficial skin infections. *J Hyg (Lond)*, 56: 238, 1958.
18. PARKER, M. T. and SIMMONS, L. E.: The inhibition of *Corynebacterium diphtheriae* and other Gram-positive organisms by *Staphylococcus aureus*. *J Gen Microbiol*, 21: 457, 1959.
19. PARKER, M. T., TOMLINSON, A. J. H. and WILLIAMS, R. E. O.: Impetigo contagiosa. The association of certain types of *Staphylococcus aureus* and of *Streptococcus pyogenes* with superficial skin infections. *J Hyg (Lond)*, 53: 458, 1955.
20. REPORT: Serotypes of *Streptococcus pyogenes*: their relative prevalence in England and Wales, 1952-1956. *Mon Bull Min Hlth Lab Serv*, 16: 163, 1957.
21. — International Committee on Phage Typing of Staphylococci. Minutes of 2nd Meeting. *Int Bull Bact Nomencl Taxon*, 9: 115, 1959.
22. SPITTLEHOUSE, K. E.: Phage-types of *Staphylococcus pyogenes* isolated from impetigo and sycosis barbae. *Lancet*, II: 378, 1955.
23. SWIFT, H. F., WILSON, A. T. and LANCEFIELD, REBECCA, C.: Typing group A haemolytic streptococci by M precipitation reactions in capillary pipettes. *J Exp Med*, 78: 127, 1943.
24. TACHAU, P.: The bacteriology of impetigo contagiosa. *Brit J Derm*, 50: 113, 1938.
25. WILLIAMS, R. E. O.: Epidemic staphylococci. *Lancet*, I: 190, 1959.

Received May 3, 1960  
Accepted June 15, 1960

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## Sensitivity of *Listeria Monocytogenes in vitro* to Different Antibiotics and Chemotherapeutics

by K. G. NYSTRÖM and K.-A. KARLSSON

During the period Jan. 1, 1948, to April 30, 1960, 177 *Listeria monocytogenes* (L.m.) strains have been isolated and collected in our Institute. In view of the increasing interest in listeriosis in Sweden we have studied a number of these strains with respect to their sensitivity *in vitro* to various antibiotics and chemotherapeutics. The classification of antibacterial substances according to origin is not considered necessary in modern medical terminology.

As has been pointed out, *inter alia*, by Ericsson & Svartz-Malmberg (3) the antibacterial spectra to antibiotics are not constant but vary from one time and one environment to another. For this reason it is of interest to study a large number of bacterial strains of the same species in order to be able to note any variations that occur in their degree of sensitivity to different antibiotics.

### Survey of Literature

For general information on animal listeriosis in Sweden the reader is referred to Nilsson & Karlsson (6). So far as we have been able to discover from the available literature, apart from Linzenmeier & Seeliger's survey (5) of the sensitivity to

antibiotics of 25 L.m. strains, no large number of these strains has been studied at any one time by the same method. On the other hand, there are numerous reports on the sensitivity of single strains to different antibiotics. Seeliger (7) summarizes the results to the effect that the tetracyclines (chlortetracycline, oxytetracycline, tetracycline) show the greatest antibiotic effect, followed by the sulphonamide compounds, the latter being most active in combination with penicillin. Resistance quickly develops against streptomycin. The use of chloramphenicol and erythromycin appears to give promising results.

Cronin & Moran (1) have reported that 9 L.m. strains investigated are sensitive to low concentrations of nitrofurantoin compounds *in vitro*.

### Material and Method

Of 177 L.m. strains isolated at the Swedish State Veterinary Medical Institute from 15 animal species, a study has been made of 86 strains (Table 1).

After the primary isolation the strains were passed at regular intervals and kept suspended in a physiological salt solution at  $-20^{\circ}\text{C}$ .

Twenty-four of the strains investigated

TABLE 1. *Listeria monocytogenes* isolated at the State Veterinary Medical Institute 1/1/1948-30/4/1960.

Animal species	No. of isolations	No. of strains tested
Poultry	124	54
Chinchilla	20	15
Hare	9	7
Cattle	5	1
Sheep	3	3
Rabbit	3	1
Roe deer	3	1
Fox	2	—
Pig	2	1
Fallow deer	1	1
Turkey	1	—
Cat	1	—
Partridge	1	1
Vole	1	—
Wildduck	1	1
Total	177	86

have been earlier serotyped by Dr. H. Seeliger in Bonn, all being found to belong to Serotype 1.

The sensitivity tests were performed by an agar diffusion technique described by Ericsson *et al.* (2, 3, 4). The strains were denoted as "sensitive", "fairly sensitive", "slightly sensitive" and "resistant". These designations correspond to Groups I, II, III and IV. The clinical evaluations of these four groups, as proposed by Ericsson *et al.*, are:

I. "Sensitive" (likely to yield to therapy in general infection; ordinary dosage).

II. "Fairly sensitive" (likely to yield to therapy in general infection; high dosage).

III. "Slightly sensitive" (likely to yield to therapy in organ in which the agent may be concentrated, e.g. certain urinary infections).

IV. "Resistant" (therapeutic effect unlikely).

This was the basis of evaluation for nine of the antibiotics tested. The *polymyxins*, however, come into a different category.

Owing to their high molecular weight and other physicochemical properties they have a slow rate of diffusion. Ericsson & Sartz-Malmberg (3) therefore advise against using the quantitative variant of the disc method for this antibiotic.

*Neomycin* and *bacitracin* are characterized by their toxicity, and with oral therapy effective concentrations are not possible except in the intestinal contents. Parenteral application (e.g. in urinary infections) often involves a risk and should be reserved for treatment when especially sensitive strains are present. For these reasons Group I was excluded when evaluating the results with these agents.

*Novobiocin* cannot be concentrated in the urine. For this antibiotic, therefore, Group II should be directly followed by Group IV.

*Nitrofurantoin*, on the other hand, attains satisfactory therapeutic concentrations only in the urinary tract. Quantitative determination of nitrofurantoin is therefore superfluous for bacteria of Groups I and II. But for the clinician all strains which are inhibited by a Group III nitrofurantoin concentration are fully suited for therapy.

## Results and Discussion

The distribution of sensitivity within the group of L.m. strains investigated, expressed in per cent, is shown in Table 2. As is seen, the tetracyclines (chlortetracycline, oxytetracycline, tetracycline), erythromycin and streptomycin are most effective against L.m. *in vitro*.

About 40 per cent of the investigated strains show resistance to sulphonamide, a higher figure than reported by Linzenmeier & Seeliger (5). It is also apparent from Figs. 1 and 2 that there is no essential difference in resistance referable to the species of animal from which the strains emanate.

As regards the tetracyclines, the present

Group

Charac

Sulfaiso

Penicilli

Erytrom

Oleando

Novobio

Streptom

Chlortet

Oxytetra

Tetracyc

Chloram

Bacitrac

Neomyc

Polymyx

Nitrofur

Fig. 1.  
strains

results

liger's

biotics

experie

cycline

reduc

cases

TABLE 2. Antibiotic sensitivity of *Listeria* strains expressed in per cent.

Group . . .	I	II	III	IV
Characterization . . .	"Sensitive"	"Fairly sensitive"	"Slightly sensitive"	"Resistant"
Sulfaisodimidine	20.9	20.9	17.5	40.7
Penicillin	4.7	74.4	20.9	0
Erythromycin	63.9	34.9	1.2	0
Oleandomycin	19.8	70.9	9.3	0
Novobiocin	4.7	52.3	See text	43.0
Streptomycin	94.1	4.7	1.2	0
Chlortetracycline	81.4	18.6	0	0
Oxytetracycline	74.4	25.6	0	0
Tetracycline	79.1	20.9	0	0
Chloramphenicol	12.8	83.7	3.5	0
Bacitracin	See text	0	1.2	98.8
Neomycin	See text	98.8	0	1.2
Polymyxin	0	0	0	100
Nitrofurantoin	See text	See text	76.7	23.3

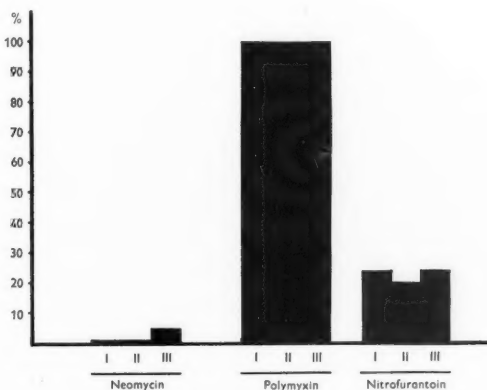


Fig. 1. Resistant strains of *Listeria monocytogenes* expressed as percentage of total number of strains isolated from various species. I, chickens (54 strains); II, chinchilla (15 strains); III, other species (17 strains).

results are in close accordance with Seeliger's data (7) on the effect of these antibiotics *in vitro* and *in vivo*. Practical experience shows treatment with tetracyclines to be of great value, having reduced the mortality even among severe cases of listeriosis. This is only on condi-

tion, however, of early administration of therapy, maximum dosage and adequate period of treatment with respect to the risk of recrudescence.

About 21 per cent of the L.m. strains investigated were "fairly sensitive" to tetracyclines. This emphasizes the im-

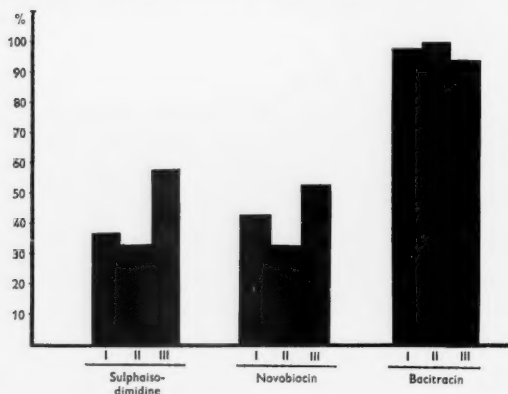


Fig. 2. Resistant strains of *Listeria monocytogenes* expressed as percentage of total number of strains isolated from various species. I, chickens (54 strains); II, chinchilla (15 strains); III, other species (17 strains).

portance of deciding on the most beneficial therapy in every case by means of sensitivity tests *in vitro*.

### Summary

The sensitivity of 14 antibiotics and chemotherapeutics to 86 strains of *Listeria monocytogenes* isolated from 15 animal

species was studied by means of an agar diffusion technique. The tetracyclines (chlortetracycline, oxytetracycline, tetracycline), erythromycin and streptomycin proved to be clearly superior to other antibiotics tested *in vitro*.

No difference in resistance was found referable to the species of animal from which the strains emanated.

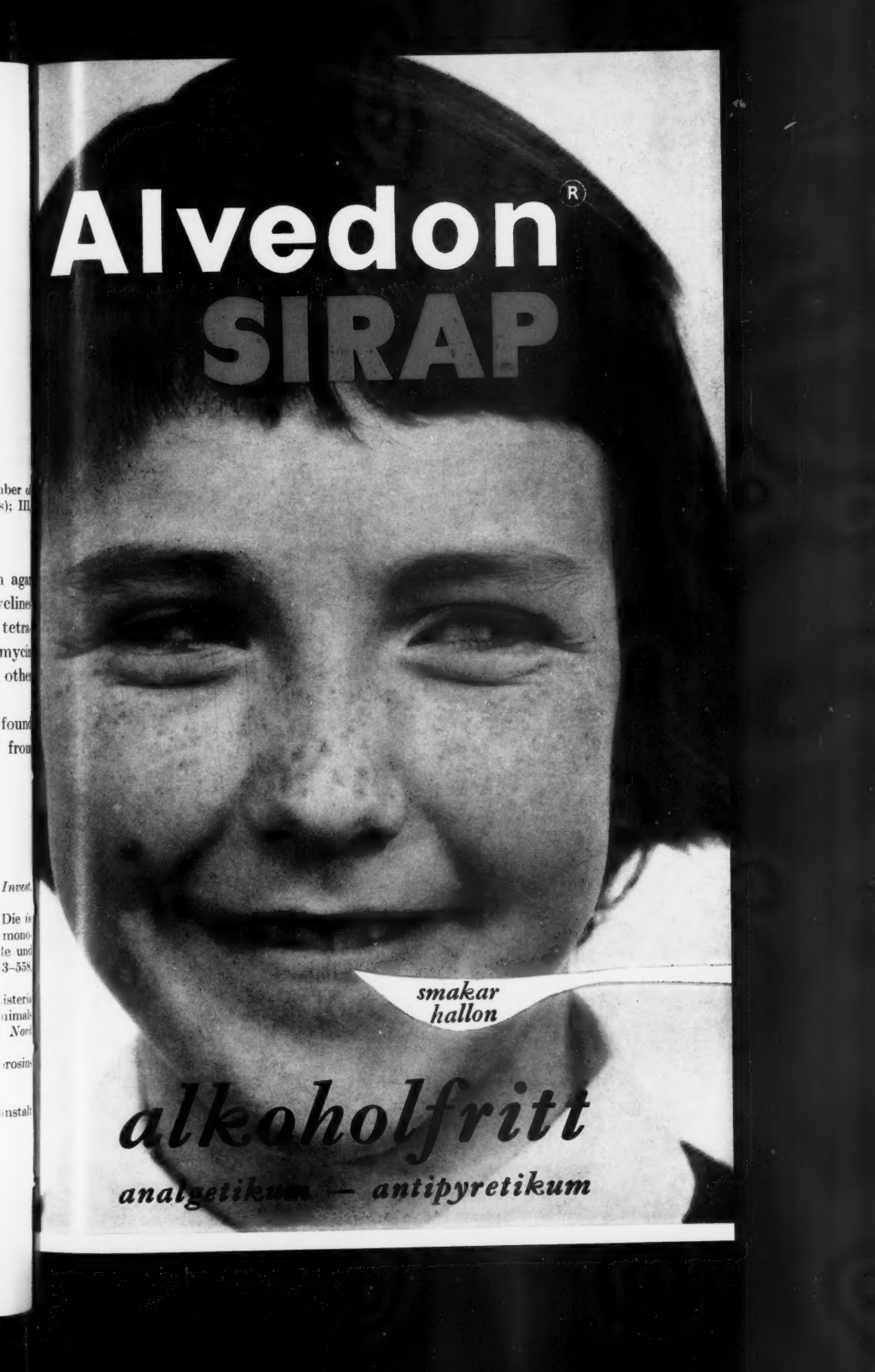
### References

1. CRONIN, M. T. I. and MORAN, A. B.: A note on the *in vitro* sensitivity to nitrofurans of *Listeria monocytogenes*. *Vet Med*, 8: 381-382, 1957.
2. ERICSSON, H., ERLANDSSON, P., TUNEVALL, G. and WALLMARK, G.: Bestämning av bakteriers kemoresistens. *Svensk Läkartidn*, 50: 476-481, 1953.
3. ERICSSON, H. and SVARTZ-MALMBERG, G.: Determination of bacterial sensitivity *in vitro* and its clinical evaluation. *Antibiot Chemother (Basel)*, 6: 41-77, 1957.
4. ERICSSON, H.: Rational use of antibiotics in hospitals. *Scandinav J Clin Lab Invest*, 12: Suppl. 50, 1960.
5. LINZENMEIER, G. and SEELIGER, H.: Die *in vitro*-Empfindlichkeit von *Listeria monocytogenes* (Pirie) gegen Sulfonamide und Antibiotica. *Zbl Bakt (Orig)*, 160: 543-558, 1953/54.
6. NILSSON, A. and KARLSSON, K.-A.: *Listeria monocytogenes* isolations from animals in Sweden during 1948 to 1957. *Nord Vet Med*, 11: 305-315, 1959.
7. SEELIGER, H.: *Listeriose*. Johann Ambrosius Barth Verlag, Leipzig 1958.

Received Aug. 9, 1960

Accepted Aug. 29, 1960

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## SIRAP

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## Sammansättning

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 Constit. .... q.s.ad 100 ml  
 F.syr.

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Feber vid förkylningssjukdomar  
 Tandvärk  
 Huvudvärk  
 Reumatiska smärtor

## Dosering

Barn:

0—1 år: 1/2 doseringssked 3—4 ggr dagl.  
 1—4 år: 1/2—1 doseringssked 3—4 ggr dagl.  
 4—8 år: 2 doseringsskedar 3—4 ggr dagl.  
 8—12 år: 2 doseringsskedar 3—4 ggr dagl.  
 12—16 år: 3 doseringsskedar 3—4 ggr dagl.  
 1 doseringssked = 5 ml = 120 mg Alvedon

## Förp. och pris

Flaska à 100 ml ..... 3:25  
 ” ” 1000 ” ..... 16:00  
 Doseringssked medföljer.

## Litteratur

1. Cornely, D. A. and Ritter, J. A.: JAMA 160, 1219 1956.
2. Coursin, D. B. and Kurtz, C. H.: Amer. Pract. Digest. Treat. 8, 1415, 1957.



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## Nocturnal Enuresis in Twins<sup>1</sup>

### II. Urethro-Cystographic Examinations

by BERTIL HALLGREN†, HARRY LARSSON and ULF RUDHE

#### Introduction

The aim of the present investigation is to study by means of micturition urethro-cystography whether any association between nocturnal enuresis and changes in the lower urinary tract can be shown, and if so, to consider the nature of the relationship. For this purpose a series of enuretic twin children and their partners were selected for investigation. The effective bladder capacity will also be examined.

In the present study, nocturnal enuresis is defined as repeated involuntary micturition during sleep after the fourth year of life. The definition does not include cases of urinary incontinence caused by any gross organic lesion in the urinary tract, the nervous system or by systemic diseases, e.g. ectopic ureters, lesions of the spinal cord, diabetes insipidus. When the enuretic child had been dry for about one year or more and had not begun to wet again, the enuresis is referred to as previous enuresis.

Opinions differ as to the causal signi-

ficance of urinary tract disorders in enuresis. Several investigators who have conducted special examinations of the lower urinary tract in enuretics state that they have found organic changes of different kinds in about half the cases—for references see *inter alia* Kjellberg, Ericsson & Rudhe (6) and Hallgren (4).

Kjellberg *et al.* examined a consecutive series of 598 enuretic children by urethro-cystography during micturition, using a specially devised method. They could not show any specific organic changes, but found changes described as pathological in 21 per cent of the children. In 80 per cent of these cases, pathological conditions were also observed by endoscopy, usually in the form of trigono-urethritis. The changes were more frequent in the group in which nocturnal enuresis was complicated with diurnal enuresis. It was particularly emphasized that no obvious cause and effect relationship existed between such findings and the occurrence of enuresis.

Giertz & Lindblom (2), examining urethro-cystographically 11 adult patients with nocturnal enuresis, stressed that the posterior urethra, especially at the level of the external sphincter, was particularly wide during micturition.

Some authors have stressed that a proportion of the children with enuresis have a

<sup>1</sup> The investigation was carried out with the aid of a grant from the Swedish Medical Research Council.

TABLE 1. *Distribution of the material by age, zygosity and sex.*

Age (years)	MZ pairs			DZ pairs			Total		
	□□	○○	Total	□□	○○	Total	□□	○○	Total
7	1	—	1	2	—	2	3	—	3
8	2	1	3	9	2	11	11	3	14
9	1	3	4	7	—	7	8	3	11
10	2	1	3	4	1	5	6	2	8
11	—	—	—	1	1	2	1	1	2
12	—	—	—	1	1	2	1	1	2
Total	6	5	11	24	5	29	30	10	40

lower effective bladder capacity than other children—*cf. inter alia* McLellan (7), Stalker & Band (10), Nash (8), Vulliamy (12). There appear, however, to be no studies with adequate statistical comparisons between groups of enuretics and controls. Poulton & Hinden (9) reported a functional bladder capacity below the norm in 12 out of 200 (6 per cent) children with enuresis. The normal values were based on the findings in 18 "normal" children. An imperative need to empty the bladder when its capacity was reached was reported in a proportion of cases by *e.g.* McLellan (7) and Stockwell & Smith (11). On the other hand, Kjellberg, Ericsson & Rudhe (6) making cystometric investigations did not find any specific pathological features.

### Material

The present cases were selected from a representative series of twins consisting of 911 pairs—*cf.* Hallgren (5). The present material comprises all same-sexed twin pairs in which at least one of the partners suffered from persistent nocturnal enuresis, *viz.* 40 pairs including 49 affected individuals, the *propositi*. The distribution of the cases by age, zygosity and sex when examined appears in Table 1.

Two pairs originally selected had to be excluded, because the X-ray films in, respectively, one and both partners were not of good enough quality to permit a detailed scrutiny.

A third pair was excluded because the

twin who wetted had a hypospadias with pronounced stenosis of the external meatus. He did not cease to wet after meatotomy, but for practical reasons a new roentgenological examination of both twins could not be arranged.

Finally, 7 pairs (13 per cent) had to be excluded because, for various reasons, the mother was not willing to cooperate in the urethro-cystographic examination. The comprehensive case histories that were available do not indicate that these pairs represent a selection in respect of any relevant factors.

When making intra-pair comparisons, those 9 pairs in which both partners were *propositi*, were excluded. Such comparisons are thus made on the basis of 31 pairs. In 6 of these pairs the twin partner of the *propositus* had a previous nocturnal enuresis.

The enuresis was primary in all but two cases, *i.e.* the child had no dry period before the age of 4 lasting continuously for approximately one year or more. A further three cases had a dry period after the age of 4. In 12 cases nocturnal enuresis was complicated with diurnal enuresis. The majority of the *propositi*, 43 out of 49 (88 per cent), had wetted once a night or more during a long period. The other *propositi* had wetted on an average once or several times a week. At the time of examination 18 cases wetted almost once a night or more.

The advantages of investigating a twin series are obvious. Monozygotic (MZ) twins, being genetically identical, offer unique opportunities of studying the effect of non-

genetic factors. Unfortunately, as regards nocturnal enuresis the degree of concordance is so high that it is difficult to obtain a sufficiently large series of discordant monozygotic twin pairs for statistical analysis. However, dizygotic (DZ) twins also are well adapted to intra-pair comparisons, since certain fundamental non-genetic factors can be kept under control, *e.g.* age and environmental factors both within and outside the family.

### Methods

The subjects were examined by *urethro-cystography during micturition* by the methods described by Kjellberg *et al.* (6). Both twins in a pair were examined on the same day, 30 of them at the Roentgenological Department, Paediatric Clinic, Karolinska Sjukhuset, Stockholm, and 10 at the Roentgenological Department, Children's Hospital, Gothenburg. The former subjects, *propositi* as well as partners, were admitted to the paediatric clinic as in-patients in connection with the examinations; the subjects from Gothenburg were examined as out-patients. All films were finally scrutinized by the same investigator (U.R.), who did not know which of the twin partners was enuretic.

The *bladder capacity* was tested only on the subjects investigated at the Karolinska Sjukhuset. It was determined as the volume of contrast medium (non-irritant barium suspension) that the children could tolerate, when the medium was injected in the bladder. This means the effective bladder capacity during an environmental strain which is equal for both partners.

Cystometric investigations may give additional information. Such a procedure was, however, difficult to carry out for practical reasons, as the examinations had to be made as a part of the routine examinations of the roentgenological department. Since the effective bladder capacity was considered to be of most interest for the present study, we refrained from cystometry.

The *determination of zygosity* was made on the basis of anthropological criteria, blood-groups and taste ability—*cf.* Hallgren (5).

In the statistical analysis<sup>1</sup> the following methods were employed. (For further details *cf. e.g.* Dixon & Massey (1).)

When testing the hypothesis that a number of samples are taken from a homogeneous population, and when testing the agreement between an observed and an expected distribution, we used the  $\chi$ -test. Where the number of subjects in any of the groups to be compared is less than 10, we have refrained from making statistical analyses.

The standard error of the mean of the differences between paired observations,  $\varepsilon_{\bar{d}}$ , was obtained with the formula

$$\varepsilon_{\bar{d}} = \frac{\sigma}{\sqrt{n}},$$

where  $\sigma$  is the standard deviation, and  $n$  is the number of pairs of observations.

The standard deviation,  $\sigma$ , was calculated from the formula

$$\sigma = \sqrt{\frac{\sum (d - \bar{d})^2}{n - 1}},$$

where  $(d - \bar{d})$  is the deviation of a difference from the mean of the differences, and  $n$  is the number of pairs of observations.

When making intra-pair comparisons with regard to the occurrence of a certain quality a sign-test was used—*cf.* Dixon & Massey (1957; Table 10a, p. 417). Only those pairs were considered in which there was an intra-pair difference in respect of the quality.

A difference is considered

significant \* if  $0.01 < p \leq 0.05$

significant \*\* if  $0.001 < p \leq 0.01$

significant \*\*\* if  $p < 0.001$

Unfortunately the number of monozygotic twins was too small for separate statistical analyses. Therefore, monozygotic and dizygotic twins were considered together in the calculations. A separate analysis of the individual monozygotic pairs was also made.

<sup>1</sup> Mr Staffan Ekblom, B.Sc., of the Statistical Research Group of the University of Stockholm, rendered us valuable help in the statistical treatment of the material.

TABLE 2. *Urethro-cystographic deviations in the lower urinary tract in twins with and without persistent nocturnal enuresis; distribution by zygosity and sex.*

Deviation	Propositus									Partner								
	MZ			DZ			Total			MZ			DZ			Total		
	□	○	Total	□	○	Total	□	○	Total	□	○	Total	□	○	Total	□	○	Total
Deficient relaxation of external sphincter	—	4	4	1	2	3	1	6	7	—	1	1	—	—	—	—	—	—
Deficient widening of bladder neck	—	—	—	2	—	2	2	—	2	—	—	—	2	—	2	2	—	2
Transverse fold in posterior urethra	—	—	—	2	—	2	2	—	2	—	—	—	—	—	—	—	—	—
Minimal reflux to ureter	1	—	1	1	—	1	2	—	2	—	1	1	1	—	1	1	1	1
Total: With deviation	1	4	5	6	2	8	7	6	13	—	1	1	3	—	3	3	3	3
Without deviation	7	4	11	20	5	25	27	9	36	4	1	5	19	3	22	23	4	4
Total	8	8	16	26	7	33	34	15	49	4	2	6	22	3	25	26	7	7

## Results

### *Urethro-cystographic examinations*

Severely pathological changes were not found in any subject. In the urethro-cystographic examinations, interest was focused on (1) deviations of a functional or anatomical nature disclosed during micturition, (2) width of urethra, particularly the posterior part (3) effective capacity of the bladder

The observed deviations consisted of a) deficient relaxation of the external sphincter during micturition with some dilatation of the urethra above this area, b) deficient widening of the bladder neck, c) the presence of a non-obstructive transverse fold in the posterior urethra at the level of the verumontanum, d) ureteral reflux (only intramurally of the bladder).

The occurrence of the *different deviations* is shown in Table 2. The frequency of deviations is approximately twice as high in the group of cases with persistent nocturnal enuresis, *i.e.* the propositi, 13 out of 49 (27 per cent), than in the group of

their partners, 4 out of 31 (13 per cent). The difference is, however, not statistically significant.

There is no significant sex difference in respect of the frequency of deviations, in the group of propositi the figures being 7 out of 34 and 6 out of 15, respectively, in that of partners 3 out of 26 and one out of 5. The number of observations is, however, small.

In the frequency of deviations there is fairly good agreement between the group of MZ twins and the group of DZ twins.

"Pathological changes" are reported to be more frequent when nocturnal enuresis is complicated with diurnal enuresis. A comparison was, therefore, also made between the group of cases with persistent nocturnal and diurnal enuresis and the group of cases with persistent nocturnal enuresis only. No significant difference between the groups emerged, the frequencies being 5 out of 12 and 8 out of 37, respectively. Again, however, the number of observations is small.

TABLE 3. *Intra-pair differences between twins with persistent nocturnal enuresis and their partners in deviations of the lower urinary tract.*

Propositus	Partner	MZ			DZ			Total		
		□	○	Total	□	○	Total	□	○	Total
Deviation	Deviation	—	1	1	2	—	2	2	1	3
Deviation	No deviation	1	—	1	2	1	3	3	1	4
No deviation	Deviation	—	—	—	1	—	1	1	—	1
No deviation	No deviation	3	1	4	17	2	19	20	3	23
Total		4	2	6	22	3	25	26	5	31

In order to make use of the advantage of the series being a twin sample an intra-pair comparison was made. Since the number of observations is comparatively small, the deviations were registered without regard to type of change. It can be seen from Table 3 that in 5 pairs one of the twins had a deviation, the other not. In 4 out of these 5 cases the twin showing deviation was the propositus. The number of observations is too small to permit a statistical analysis.

The monozygotic twins were discordant in respect of deviations in three instances.

In two cases, one of them being a propositus, the deviation in question was ureteral reflux. One of the cases with reflux voided very frequently during the day, the other was suspected of having had an infection of the lower urinary tract at the age of two. No other somatic factors of possible importance were found. One of the cases was considered to be emotionally somewhat less stable than his partner, the other to be more stable than his partner, but the differences were small. None of them had nervous problems.

In the third case, a proposita, the deviation in question was deficient relaxation of the external sphincter during micturition. This girl, as well as her twin sister who also had persistent nocturnal enuresis, wetted very frequently during the day. No somatic diseases of possible importance were found. The girl with deficient relaxation was con-

sidered to be somewhat less stable than her partner, but had no nervous problems.

#### *Width of urethra during micturition*

By making intra-pair comparisons we could avoid the subjective decision of what is and what is not pathological in respect of the width of the urethra, i.e. its posterior portion and particularly at the level of external sphincter, during micturition.

It appears from Table 4 that an intra-pair difference in the width of the posterior urethra was considered to exist in 10 instances, in 6 of which the propositus had the greater width. The distribution agrees rather well with that to be expected if there were no relationship between the calibre of the posterior urethra during micturition and nocturnal enuresis.

In 5 monozygotic pairs there was an intra-pair difference in the width of the posterior urethra during micturition; in three of these pairs both twins were propositi; in the two other pairs the twin who did not wet had the wider urethra during micturition.

Disease of the urinary tract, viz. a cystopyelitis at the age of 7 (two years before the present examination), occurred in one case, who had a greater width than her twin sister. Diurnal frequency of micturition and urgency occurred in two pairs, both partners in each pair having the same micturition

TABLE 4. *Intra-pair differences between twins with persistent nocturnal enuresis and their partners in the width of the posterior urethra.*

Width of posterior urethra	MZ			DZ			Total		
	□	○	Total	□	○	Total	□	○	Total
Wider in propositus	—	—	—	6	—	6	6	—	6
Wider in partner	2	—	2	1	1	2	3	1	4
No difference	2	2	4	15	2	17	17	4	21
Total	4	2	6	22	3	25	26	5	31

TABLE 5. *Intra-pair differences between twins with persistent nocturnal enuresis and their partners in the width of the urethra at the level of the external sphincter.*

Width of urethra	MZ			DZ			Total		
	□	○	Total	□	○	Total	□	○	Total
Wider in propositus	2	—	2	4	1	5	6	1	7
Wider in partner	—	—	—	10	1	11	10	1	11
No difference	2	2	4	8	1	9	10	3	13
Total	4	2	6	22	3	25	26	5	31

TABLE 6. *Mean width of urethra and intra-pair differences in the width between twins with persistent nocturnal enuresis and their partners.*

Twin pair		Number of pairs of observations	Mean width of urethra		Mean of differences
			Propositus	Partner	
MZ	□	4	6.25	7.13	—
	○	2	4.50	4.25	—
	□ + ○	6	5.67	6.17	—
DZ	□	22	6.86	6.16	—
	○	3	6.83	7.17	—
	□ + ○	25	6.86	6.28	—
Total	□	26	6.77	6.31	—
	○	5	5.90	6.00	—
	□ + ○	31	6.63	6.26	0.371 ± 0.251

symptoms of approximately the same intensity.

An intra-pair difference in the width of the urethra at the level of the external sphincter was found in 18 instances, in 7 of which the propositus had the greater width—*cf.* Table 5. The distribution does

not differ significantly from that to be expected if there were no relationship between nocturnal enuresis and width of urethra.

A difference in width is considered to exist when it amounts to 1 mm or more as measured in the films.

TABLE 7. *Intra-pair differences between twins with persistent nocturnal enuresis and their partners in bladder capacity.*

Bladder capacity	MZ			DZ			Total		
	□	○	Total	□	○	Total	□	○	Total
Smaller in propositus	1	1	2	8	1	9	9	2	11
Smaller in partner	—	—	—	5	—	5	5	—	5
No difference	2	1	3	5	—	5	7	1	8
Total	3	2	5	18	1	19	21	3	24

TABLE 8. *Mean bladder capacity and intra-pair differences in the capacity between twins with persistent nocturnal enuresis and their partners.*

Twin pair		Number of pairs of observations	Mean capacity		Mean of differences
			Propositus	Partner	
MZ	□	3	200	217	—
	○	2	145	170	—
	□ + ○	5	178	198	—
DZ	□	18	213	219	—
	○	1	150	200	—
	□ + ○	19	210	218	—
Total	□	21	211	218	—
	○	3	147	180	—
	□ + ○	24	203	213.5	10.4 ± 8.2

In respect of the width at the level of the external sphincter, there is a rather good agreement between the group of twins with persistent nocturnal enuresis and that of others — *cf.* Table 6.

#### *Bladder capacity*

The intra-pair comparison in respect of the effective bladder capacity appears in Table 7. The bladder capacity was smaller in the propositus in 11 instances and in the partner in 5.

The observed distribution does not differ significantly from that to be expected if there were no association between nocturnal enuresis and bladder capacity.

A difference in capacity is considered to exist when it amounts to 20 ml or more.

There is no significant difference in respect of the mean of differences between the group of twins with persistent nocturnal enuresis and that of others—*cf.* Table 8. The mean capacity in the two groups agrees fairly well.

There was a difference (between 50–90 ml) in the bladder capacity in 4 monozygotic pairs, in two of which both partners having persistent nocturnal enuresis. In none of the cases was there any noticeable difference between the pairs in respect of emotional stability or micturition symptoms. Nor was there in any case information on diseases of the lower urinary tract.



TABLE 9. *Intra-pair differences between twins with persistent nocturnal enuresis and their partners in the occurrence of spina bifida occulta.*

Propositus	Partner	MZ			DZ			Total		
		□	○	Total	□	○	Total	□	○	Total
Spina bifida	Spina bifida	1	—	1	2	—	2	3	—	3
Spina bifida	No spina bifida	1	—	1	3	—	3	4	—	4
No spina bifida	Spina bifida	—	—	—	4	1	5	4	1	5
No spina bifida	No spina bifida	2	2	4	13	2	15	15	4	19
Total		4	2	6	22	3	25	26	5	31

In the calculations certain possible *biases* in the interpretation of the urethro-cystographic findings were considered.

A priori, the observed deviations in the lower urinary tract as well as the width of the urethra during micturition and bladder capacity may be connected with previous or present lesions in lower urinary tract—in the present series only infections occurred, *e.g.* cystopyelitis—with certain micturition symptoms, *viz.* diurnal frequency of micturition (*pollakisuria*), urgency or with nervous tension during the examination. The possible influence on the results of the calculations in respect of enuresis of these biases was tested in the following way. The frequency of deviations found on urethro-cystography, the frequencies of cases with wider urethra and smaller bladder capacity than the partner in the respective groups of children with lower urinary-tract infections, psychiatric problems ("problem children"; *cf.* Hallgren (3)) and micturition symptoms were compared with the corresponding frequencies in the groups of children without urinary tract infections, psychiatric problems and micturition symptoms respectively. It emerged from the calculations that the above frequencies agreed rather well in the groups that were compared. Nor were there any significant differences in the frequencies between the group of twins which were considered by their mothers to be emotionally less stable than the respective co-twins and the group of the emotionally more stable

partners. Hence, the occurrence of lesions in the lower urinary tract, micturition symptoms and psychiatric symptoms could be disregarded in the calculations with regard to enuresis.

Furthermore, if there is any association between enuresis, on the one hand, and deviations in the lower urinary tract, width of the urethra or bladder capacity, on the other hand, it is possible that cases with a previous enuresis may disclose persistent pathological conditions. If such were the case, this would affect the result of the comparisons. However, the result is not changed if the 6 cases with previous nocturnal enuresis are excluded from the calculations. Therefore, in the calculations these 6 cases have been included in the group of non-enuretics.

### *Spina bifida occulta*

The roentgenological examination also permitted a registration of the occurrence of spina bifida occulta in the lower lumbar and the sacral regions. This anomaly, located in L<sub>5</sub> or/and S<sub>1</sub> and S<sub>2</sub>, was found in 9 out of 49 propoiti and in 8 out of 31 of their partners. The difference is not significant. The intra-pair comparison shows that in 9 pairs only one of the twins had spina bifida — *cf.* Table 9. In 4 cases it was the propositus, in 5 the partner.

From the genetic point of view it is of some interest to note that of the three

monozygotic pairs in which one or both twin-partners had spina bifida occulta, two were discordant in respect of this sign.

### Discussion

In considerations of aetiology and pathogenesis of enuresis, the twin-research method offers some fundamental advantages, in as much as it enables the investigator to keep some important relevant factors under control. The present study is based on a randomly selected series of twin children with nocturnal enuresis, using the twin partners as controls. The aim of the investigation was to ascertain whether there was any relationship between disorders of the lower urinary tract as revealed by micturition urethro-cystography and the occurrence of nocturnal enuresis. In order to avoid prejudicing influences in assessing the findings demonstrated by urethrocystography, the examiner was not informed whether the child was affected with enuresis or not. Since, in addition, possible biasing factors were especially taken into account in the calculations, it is considered that the analysis enables certain conclusions to be drawn which are presumably to some extent generally applicable to enuretic children.

The analysis has failed to reveal any significant associations between nocturnal enuresis, on the one hand, and the width of the urethra or the effective capacity of the bladder, on the other hand. Nor could any significant relationship be established between enuresis and minor functional or anatomical derangements of the lower urinary tract, i.e. deficient opening of the bladder neck or the external sphincter

or the presence of a non-obstructive transverse fold in the posterior urethra. Moreover, severely pathological changes in the lower urinary tract were not found in any case. Thus, the result does not provide any support to the assumption of some previous authors of such relationships to exist. Previous studies, however, are not based on adequately controlled series. On the other hand, the frequency of minor deviations in the lower urinary tract is, in the present series, higher, though not significantly, in the group of twins with persistent nocturnal enuresis than in the group of their partners. It is, therefore, possible that a larger series would reveal a significant relationship between such deviations and nocturnal enuresis. The present study, however, indicates that such a relationship, if it exists, is not strong, at least as far as *children* are concerned.

### Summary

1. A study by means of micturition urethro-cystography of changes in the lower urinary tract and of the effective bladder capacity in children with nocturnal enuresis was performed on the basis of a randomly selected twin series. The material comprised 40 same-sexed twin pairs in which at least one of the partners suffered from persistent nocturnal enuresis, i.e. 49 affected twins and 31 unaffected partners who were used as controls.

2. Only minor deviations in the lower urinary tract were found. The investigation failed to show any significant relationship between such deviations and nocturnal enuresis.

3. There seems to be no relationship between nocturnal enuresis and the width of the posterior urethra during micturition.

4. The statistical analysis indicates that there is no difference in the effective bladder capacity between children with per-

sistent nocturnal enuresis and other children.

5. There seems to be no significant relationship between nocturnal enuresis and the occurrence of spina bifida occulta in the lower lumbar and sacral regions.

### References

1. DIXON, W. J. and MASSEY, F. J.: Introduction to Statistical Analysis. New York 1957.
2. GIERTZ, G. and LINDBLOM, K.: Urethro-cystographic studies of nervous disturbances of the urinary bladder and the urethra. A preliminary report. *Acta Radiol (Stockh)*, 36: 205, 1951.
3. HALLGREN, B.: Enuresis. A clinical and genetic study. *Acta Psychiat Scand, Suppl*, 114: 1957.
4. — Nocturnal enuresis; aetiologic aspects. *Acta Paediat, Suppl*, 118: 66, 1959.
5. — Nocturnal enuresis in twins. I. Method and material. *Acta Psychiat Scand*, 35: 73, 1960.
6. KJELLBERG, S. R., ERICSSON, N. O. and RUDHE, U.: The Lower Urinary Tract in Childhood. Almqvist & Wiksell and Year Book Publish., Stockholm and Chicago, 1957.
7. McLELLAN, F. C.: The Neurogenic Bladder. Charles C. Thomas, Springfield 1939.
8. NASH, D. F. E.: The development of micturition control with special references to enuresis. *Ann Roy Coll Surg England*, 5: 319, 1949.
9. POULTON, E. M. and HINDEN, E.: The classification of enuresis. *Arch Dis Child*, 28: 392, 1953.
10. STALKER, H. and BAND, D.: Persistent enuresis: a psychosomatic study. *J Ment Sci*, 92: 324, 1946.
11. STOCKWELL, L. and SMITH, C. K.: Enuresis. A study of causes, types and therapeutic results. *Amer J Dis Child*, 59: 1013, 1940.
12. VULLIAMY, D.: The day and night output of urine in enuresis. *Arch Dis Child*, 31: 439, 1956.

Received June 28, 1960

Accepted July 1, 1960

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## The Vitamin B<sub>1</sub>, B<sub>2</sub> and C Content in Urine of Premature and Fullterm Newborn Infants

by M. DAHL, T. MARKKANEN<sup>1</sup> and T. VANHA-PERTTULA

Reports in the literature on the requirements and excretion of vitamin B<sub>1</sub>, B<sub>2</sub> and C in premature and full-term newborn infants are contradictory to some extent. The purpose of this investigation was to study the excretion of these vitamins in the urine of premature and newborn infants, and, in the latter group, to compare it with the infants' general health and the mothers' pre-confinement state.

### Material and Methods

The daily urine output of 15 premature and 24 newborn full-term infants was collected on three consecutive days by the Coloplast bag method. Daily studies were done on urine samples which were acidified and kept in darkness. Because of technical difficulties in collecting urine samples, all infants were boys. The urine samples were taken on the three first days of life at the Maternity Hospital.<sup>2</sup> Other specimens were obtained from the Department for Premature Infants. At the time of investigation,

the premature infants were 3-84 days old and weighed under 2400 g. All required treatment for prematurity. These infants were given a diet of human milk, sugar and water, until their weight reached approximately 2100 g. After that, the diet was gradually changed to a formula consisting of  $\frac{2}{3}$  cow's milk and  $\frac{1}{3}$  water, with 2% wheat flour and 5% sugar. From the age of 3 weeks, the premature infants received 25 mg/day of vitamin C, and from the age of 4 weeks, 2 drops of Jekovit (1 drop contains approximately 550 I.U. vitamin A, and approximately 700 I.U. vitamin D) twice daily. The micro-biological analyses of thiamine in the urine samples were performed according to Sarett & Cheldelin's method (22), and the riboflavin analyses according to Snell & Strong's method (29). For chemical determination of ascorbic acid, the method of Schaffert & Kingsley (23) was followed.

### Results

The results are presented in Tables 1 and 2.

In premature infants aged less than 3 weeks, the average vitamin C excretion was 1.78 mg per cent. In those over 3 weeks receiving 25 mg/day of vitamin C, the average excretion was 1.16 mg per cent.

<sup>1</sup> Aided by grants from the Suomen Kulttuurirahasto, Helsinki, Finland.

<sup>2</sup> We thank Professor S. Parviainen, the head of the Department of Obstetrics, University of Turku, Finland, for the permission to collect the samples from the newborn infants.

TABLE 1. *Vitamin B<sub>1</sub>, vitamin B<sub>2</sub> and vitamin C excretion in premature infants.*

Case	Age <sup>a</sup> in days	Weight in grams	Vitamin B <sub>1</sub> excretion in r%				Vitamin B <sub>2</sub> excretion in r%				Vitamin C excretion in mg %			
			1. day	2. day	3. day	Av- erage	1. day	2. day	3. day	Av- erage	1. day	2. day	3. day	Av- erage
1	3- 5	2400	<1	<1	<1	<1	0.0	—	0.0	6.0	1.35	—	1.25	1.30
2	4- 6	2900	<1	<1	<1	<1	0.0	11.0	18.0	9.7	2.60	8.65	1.35	4.20
3	5- 7	1800	<1	<1	<1	<1	0.0	0.0	16.0	5.3	—	0.75	0.98	0.87
4	7- 9	1600	<1	<1	<1	<1	17.0	17.0	20.0	18.0	3.25	1.30	1.28	1.94
5	9-11	1500	<1	<1	<1	<1	0.0	0.0	0.0	0.0	1.65	0.20	0.50	0.78
6	12-14	2200	<1	<1	<1	<1	5.0	0.0	0.0	1.7	4.90	1.45	1.55	2.63
7	14-16	1500	<1	<1	<1	<1	0.0	0.0	0.0	0.0	—	—	0.77	0.77
8	21-23	2100	<1	<1	—	<1	22.0	35.0	—	28.5	1.90	—	—	1.90
9	29-31	2100	<1	<1	<1	<1	12.0	12.0	12.0	12.0	4.95	2.25	1.05	2.42
10	32-34	1900	<1	<1	<1	<1	25.0	25.0	11.0	20.3	0.00	0.10	1.75	0.62
11	32-34	2000	<1	<1	<1	<1	22.0	15.0	35.0	20.7	0.75	1.90	—	1.33
12	36-38	2100	<1	<1	<1	<1	52.0	52.0	52.0	52.0	0.05	0.60	0.25	0.30
13	40-42	2200	7.0	4.0	4.0	5.0	25.0	30.0	25.0	26.7	0.45	0.55	1.60	0.87
14	41-43	1800	<1	<1	<1	<1	60.0	52.0	68.0	60.0	0.55	0.00	1.05	0.53
15	82-84	2100	<1	<1	<1	<1	5.0	0.0	15.0	6.7	—	0.67	2.05	1.36
										17.4	1.45			
										± 4.7	± 0.75			

TABLE 2. Vitamin B<sub>1</sub>, vitamin B<sub>2</sub> and vitamin C excretion in newborn infants.

Mothers' state of health (condition)				Birth weight in g	Vitamin B <sub>1</sub> excretion in p %			Vitamin B <sub>2</sub> excretion in p %			Vitamin C excretion in mg %						
Case	Partus no.	Hgb g %	WeightLength in kg in cm		1. day <sup>a</sup>	2. day	3. day	Av. excretion	1. day <sup>a</sup>	2. day	3. day	Av. excretion	1. day <sup>a</sup>	2. day	3. day	Av. excretion	
Complicating diseases																	
1	I	11.8	61.5	161	3050	8.0	4.0	2.0	4.7	0.0	5.0	0.0	1.7	1.90	1.30	0.30	1.37
2	II	13.7	61.0	167	3130	28.0	<1.0	5.0	11.0	11.0	5.0	5.0	7.0	3.30	0.60	1.20	1.70
3	I	15.4	66.1	163	3220	<1.0	6.0	3.0	3.0	55.0	42.0	10.0	35.7	1.35	—	1.70	1.52
4	I	13.3	63.0	160	3250	16.0	24.0	2.0	14.0	12.0	6.0	6.0	6.0	4.00	1.15	1.30	5.15
5	III	13.7	71.0	160	3430	8.0	4.0	—	6.0	40.0	12.0	—	26.0	2.85	1.00	—	1.92
6	I	13.7	67.5	158	3550	2.0	6.0	<1.0	2.7	33.0	0.0	0.0	11.0	2.00	2.00	1.15	1.72
7	II	11.8	76.5	167	3570	<1.0	14.0	20.0	13.0	18.0	25.0	5.0	16.0	2.45	9.00	12.35	7.93
8	I	10.5	72.5	169	3600	2.0	2.0	1.0	1.3	52.0	20.0	0.0	24.0	10.80	2.15	4.20	5.72
9	III	11.5	88.0	155	3650	4.0	16.0	24.0	14.7	44.0	12.0	10.0	22.0	14.90	2.20	8.00	8.37
10	III	12.2	67.0	162	3650	<1.0	<1.0	<1.0	<1.0	0.0	0.0	0.0	0.0	1.15	1.55	2.75	1.82
11	IV	15.0	75.0	168	3670	—	2.0	<1.0	1.5	—	0.0	0.0	0.0	—	3.90	2.15	3.02
12	I	11.8	85.0	155	3800	6.5	2.0	<1.0	2.8	68.0	20.0	0.0	29.3	0.60	1.65	3.50	1.99
13	I	14.5	74.0	162	3900	6.5	13.0	2.0	7.2	52.0	20.0	0.0	24.0	—	9.50	3.05	6.28
14	V	13.7	81.5	162	3900	6.0	2.0	—	4.0	28.0	4.0	—	16.0	1.70	—	—	1.70
15	VII	13.7	102.0	165	3920	28.0	28.0	20.0	26.0	60.0	5.0	5.0	23.0	3.35	2.85	3.60	3.33
16	III	12.9	74.0	165	3950	4.0	11.0	—	7.5	35.0	40.0	—	37.5	3.30	3.40	4.05	—
17	I	8.7	66.9	164	4000	<1.0	3.0	—	2.0	35.0	16.0	—	25.5	3.60	5.65	—	4.63
bophlebitis superf. et prof. cur. I. sin.																	
18	VIII	11.8	71.8	156	4020	30.0	12.0	3.0	15.0	75.0	15.0	0.0	28.3	3.05	3.45	0.25	2.25
19	VI	13.3	65.0	161	4040	5.0	10.0	6.0	7.0	5.0	30.0	15.0	16.7	4.00	7.30	8.10	6.47
20	I	10.5	76.7	168	4150	15.0	50.0	90.0	51.7	90.0	52.0	11.0	51.0	5.65	4.90	9.65	6.73
Ptyelonephritis gravid. Hy-pertensio gravid.																	
21	IV	11.5	67.0	161	4150	3.0	5.0	—	4.0	50.0	25.0	—	37.5	2.75	5.00	—	3.88
22	I	13.3	97.6	162	4240	9.0	<1.0	<1.0	3.7	5.0	5.0	5.0	5.0	7.85	1.50	1.10	3.48
23	I	12.5	77.4	167	4250	16.0	16.0	16.0	16.0	25.0	18.0	16.0	19.7	10.70	5.40	9.55	8.55
24	III	11.5	73.5	166	4570	6.5	—	16.0	11.2	112.5	—	12.0	62.3	10.40	—	21.40	15.90
Ptye-eclampsia gravidis. Influenza																	
8.9 10.4 11.1 9.6 39.3 16.3 4.9 21.9 4.63 4.08 5.02 4.54 ±0.68 ±2.2 ±3.1																	

<sup>a</sup> 1, 2 and 3 denote the first three days of life.

cluded in this study was good, and the congenital vitamin B and C depots may therefore be considered to represent a normal level.

The vitamin B and C excretion in the urine is generally thought to be a good index of the nutritional status. Low values are said to indicate insufficient administration of vitamin or abnormally high consumption (14).

Reports on the vitamin B requirement of the premature and newborn infants are somewhat contradictory. According to Bartam (Nelson (17)), the newborn requires 0.4 mg/day of vitamin B<sub>1</sub> and 0.6 mg/day of vitamin B<sub>2</sub>; these requirements increase rapidly in the first months of life. Evidently premature infants, according to Dunham (4), need larger amounts of vitamin B than do other newborn infants. On the other hand, Anderson (1) takes a neutral position on this question and says that feeding of vitamins B even to premature infants is not injurious. Dean & Holman (3) subjected two infants to preponderance tests. On the basis of their results, they considered the vitamin values to be entirely too high. In 1959, Smith (28) expressed this same opinion. According to the Food and Nutrition Board (1958), breast fed infants do not need additional vitamins during the first month of life. From the second month onward, the requirement of vitamin B<sub>1</sub> is 0.4 mg/day and that of vitamin B<sub>2</sub> 0.5 mg/day (5). Deficiencies of vitamin C in the diet of premature infants and bottle fed full-term infants is a generally accepted observation (24, 25, 26). Smith (28) advises administration of 25 mg/day of vitamin C to premature and bottle fed full-term infants, beginning with the second or third week of life. The Food and Nutrition Board recommends 30 mg/day of vitamin C for infants from the second month onward (5).

Even under favourable conditions, the vitamin B<sub>1</sub> and B<sub>2</sub> depots are comparatively small in the newborn.

Slobody *et al.* (27) state that in the blood of newborn infants, the average vitamin B<sub>1</sub> level was 12  $\mu$  per cent. After 5 days, the average value was only 9.6  $\mu$  per cent. Neuweiler's (18) observations show that the temporary excess disappears in the first days of life, after which the excretion of vitamins B<sub>1</sub> and B<sub>2</sub> is extremely low. In his textbook, Brock (2) writes that during the first days of life, the excretion of vitamin B<sub>1</sub> may reach 13  $\mu$  per cent, vitamin B<sub>2</sub> 140  $\mu$  per cent, and vitamin C 1.5–10.0 mg/day. Later, infants excrete 5–30  $\mu$  per cent vitamin B<sub>1</sub>, 0.2–29.0  $\mu$  per cent vitamin B<sub>2</sub> and up to 100 mg/day vitamin C. According to Hamil (7), the average vitamin B<sub>1</sub> excretion in the urine during the seventh day of life is 0  $\mu$  per cent, and vitamin B<sub>2</sub> 2  $\mu$  per cent. Knott (8) states that 7–22 week old infants excrete 3  $\mu$  per cent of vitamin B<sub>1</sub> in four hours, and the excretion of breast fed infants is less than that of the artificially fed ones. Toverud (32) reports that breast fed infants do not excrete any vitamin B<sub>1</sub> at all. This is understandable as the vitamin B<sub>1</sub> and B<sub>2</sub> levels in human milk are lower than in cow's milk (8, 9, 11, 20, 21, 32, 33, 34). In regard to vitamin C, the relationship is the opposite (9). Toverud-Stearns-Macy (34) give the following B<sub>1</sub>, B<sub>2</sub> and C levels in human and cow's milk:

	Human milk	Cow's milk
Vitamin B <sub>1</sub>	15 $\mu$ %	42 $\mu$ %
Vitamin B <sub>2</sub>	46.9 $\mu$ %	158 $\mu$ %
Vitamin C	4.4 mg %	1.9 mg %

Dunham (4) states that when sugar is added to the milk mixture, the vitamin B requirement of the organism is raised.

In this study, the general state of health of the infants' mothers was good. The values for vitamin excretion in both exceptionally stout mothers in good condition and of anemic mothers were normal. The vitamin B<sub>1</sub> excretion of an infant



delivered by a highly anemic mother (Case 17) was found to be low. The vitamin B<sub>1</sub> excretion in many other children, however, was still lower, and no distinct relationship between these occurrences can be made. Diseases complicating pregnancy, i.e. pre-eclampsia, hypertensio gravidarum and influenza, did not influence the infant's vitamin excretion either.

Comparing the results obtained in this investigation, it was observed that practically no vitamin B<sub>1</sub> excretion occurred in premature infants, regardless of age and weight. In any event, in none of the cases were vitamin B<sub>1</sub> deficiency symptoms clinically noted. These observations agree well with previously published studies, and the clinical reports that B<sub>1</sub> avitaminosis is the most common of the vitamin deficiencies in the newborn. It may be reasonable to bear in mind that infantile beriberi could appear even in this country.

The excretion values for vitamin B<sub>2</sub> in premature infants correspond completely with those given in previous reports. The danger that deficiency states would develop does not seem to be great, although considerable differences are observed between the various cases in our material. The values for vitamin C excretion are low. The observation that vitamin C excretion in premature infants over three weeks

of age, despite administration of vitamin C, is less than in younger infants, strongly supports the necessity of vitamin C administration.

Values for the excretion of Vitamin B<sub>1</sub> in the urine of newborn full-term infants remain high and relatively unchanged in the three first days of life. The average excretion values correspond to those given by Brock (2). The abundant excretion of vitamin B<sub>2</sub> agrees well with Neuweiler's (18) observations. The excretion values for vitamin C remain, on an average, equally high on the first three days of life.

The weight of the newborn at birth was not noted to have any distinct influence on the vitamin content in the urine. The same observation was made in premature infants.

### Summary

The daily excretion of vitamins B<sub>1</sub>, B<sub>2</sub> and C was studied in 24 newborn full-term infants during the three first days of life, and in 15 premature infants on three successive days of treatment. No correlation was observed between the mothers' state of health during pregnancy, the number of previous births and the presence of certain diseases, with the daily excretion of vitamin B<sub>1</sub>, B<sub>2</sub> and C in the urine of newborn full-term infants.

### References

1. ANDERSON, N. A.: Care of the premature infant. Mitchell-Nelson: Textbook of Pediatrics. Fifth Edition. Saunders, Philadelphia. 1950.
2. BROCK, J.: Biologische Daten für den Kinderarzt. Springer, Berlin. II Band: 330, 1954.
3. DEAN, R. F. A. and HOLMAN, W. I. M.: Excretion and intake of B vitamins in newborn infants. *Arch Dis Child*, 25: 292, 1950.
4. DUNHAM, E. C.: Premature Infants. Hoeber-Harper, New York. 1955.
5. Food and Nutrition Board, National Research Council: Recommended Dietary Allowances. Revised 1958.
6. HAMIL, B., MUNKS, B., MOYER, E., KAUCHER M. and WILLIAMS, H.: Vitamin C in the blood and urine of the newborn and in the cord and maternal blood. *Amer J Dis Child*, 74: 417, 1947.
7. HAMIL, B., CORYELL, M., RODERUCK, C.,

- KAUCHER, M., MOYER, E., HARRIS, M. and WILLIAMS, H.: Thiamine, riboflavin, nicotinic acid, pantothenic acid and biotin in the urine of newborn infants. *Amer J Dis Child*, 74: 434, 1947.
8. KNOTT, E. M.: Determination of vitamin B<sub>1</sub> requirement of infants by means of urinary excretion of thiamine. *Proc Soc Exp Biol Med*, 45: 765, 1940.
  9. LAWRENCE, J., HERRINGTON, B. and MAYNARD, L.: Comparative values of bovine and human milks in infant feeding. *Amer J Dis Child*, 70: 193, 1945.
  10. LEVINE, S. Z., GORDON, H. H. and MARPLES, E. I.: Defect in the metabolism of tyrosine and phenylalanine in premature infants. II. Spontaneous occurrence and eradication by vitamin C. *J Clin Invest*, 20: 209, 1941.
  11. LITCHFIELD, H. R., LICHTERMAN, J., KNOLL, I. and KURLAND, I.: Effect of yeast extract (Vitamin B complex) on growth and development of premature infants. *Amer J Dis Child*, 57: 546, 1939.
  12. LUND, C. and KIMBLE, M.: Some determinations of maternal and plasma vitamin C levels. *Amer J Obstet Gynec*, 46: 635, 1943.
  13. LUST, J. E., HAGERMAN, D. D. and VILLEE, C. A.: The transport of riboflavin by human placenta. *J Clin Invest*, 33: 38, 1954.
  14. MELNICK, D.: Vitamin B<sub>1</sub> (thiamine) requirement of man. *J Nutr*, 24: 139, 1942.
  15. NELSON, M. M., BAIRD, C. D. C., WRIGHT, H. V. and EVANS, H. M.: Multiple congenital abnormalities in the rat resulting from riboflavin deficiency induced by the antimetabolite galactoflavin. *J Nutr*, 58: 125, 1956.
  16. NELSON, M. M.: Production of congenital anomalies in mammals by maternal dietary deficiencies. *Pediatrics*, 19: Suppl. 4: 764, 1957.
  17. NELSON, W. E.: Textbook of Pediatrics. Sixth Edition. Saunders, Philadelphia-London, 1954.
  18. NEUWEILER, W.: Refer. by Dean & Holman.
  19. NITOWSKY, H. M., GOVAN, C. D. and GORDON, H. H.: Effect of hemopoietic and other agents on the hydroxyphenyluria of premature infants. *Amer J Dis Child*, 85: 462, 1953.
  20. RODERUCK, C., CORYELL, M., WILLIAMS, H. and MACY, I.: Free and total riboflavin contents of colostrum and mature human milk. *Amer J Dis Child*, 70: 171, 1945.
  21. RODERUCK, C., WILLIAMS, H., and MACY, I.: Free and total thiamine contents of colostrum and mature human milk. *Amer J Dis Child*, 70: 162, 1945.
  22. SARETT, H. P. and CHELDELIN, V. H.: Use of lactobacillus fermentum 36 for thiamine assay. *J Biol Chem*, 155: 153, 1944.
  23. SCHAFFERT, R. R. and KINGSLEY, G. R.: Rapid, simple method for determination of reduced, dehydro- and total ascorbic acid in biological material. *J Biol Chem*, 212: 59, 1955.
  24. SELLEG, J. and KING, C. G.: Vitamin C content of human milk and its variation with diet. *J Nutr*, 11: 599, 1936.
  25. SLOBODY, L., BENSON, R. and MESTERN, J.: A comparison of the vitamin C in mothers and their newborn infants. *J Pediatr*, 29: 41, 1946.
  26. — A comparison of vitamin C in mothers and their premature newborn infants. *J Pediatr*, 31: 333, 1947.
  27. SLOBODY, L., WILLNER, M. and MESTERN, J.: Comparison of vitamin B levels in mothers and their newborn infants. *Amer J Dis Child*, 77: 736, 1949.
  28. SMITH, C. A.: The Physiology of the Newborn Infant. Third Edition. Blackwell Scientific Publications, Oxford, 311-319, 1959.
  29. SNELL, E. E. and STRONG, F. M.: Microbiological assay for riboflavin. *Indust Engng Chem (Anal)* 11: 346, 1939.
  30. STEARNS, G.: Nutritional state of the mother prior to conception. *JAMA*, 169: 1655, 1958.
  31. STEVENSON, S. S.: Possible ariboflavinosis in a premature infant. *Yale J Biol Med*, 14: 403, 1943.
  32. TOVERUD, K. U.: The excretion of aneurin in pregnant and lactating women and in infants. *Z Vitaminforsch*, 10: 255, 1950.
  33. — Beretning Om De Forste 6 Ars Arbeid Ved Oslo Kommunes Helsestasjon For Mor Og Barn På Sagene (1939-1944). Fabritius and Sons, Oslo, 1945.
  34. TOVERUD, K. U., STEARNS, G. and MACY, I. G.: Maternal nutrition and child health. An interpretative review. *Bull Nat Res Coun (Wash)*, 123, 1950.
  35. VAN GELDER, D. and DARBY, F.: Congenital and infantile beriberi. *J Pediatr*, 25: 220, 1944.
  36. WARKANY, J. and NELSON, R. C.: Appearance of skeletal abnormalities in the offspring of rats reared on a deficit diet. *Science*, 92: 383, 1940.

Received June 16, 1960

Accepted July 12, 1960

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## Growth Changes in the Mongoloid Head<sup>1</sup>

by A. F. ROCHE, F. S. SEWARD and S. SUNDERLAND

There has been a rather lively discussion about the form, size and growth of the head in mongoloid children (see f.i. Benda (4, 6)). As a contribution to the study of this question this paper, which is concerned with growth changes involving the head in white Australian mongoloids, will be presented.

### Material and Methods

Our findings are based on data obtained during a study of 67 male and 81 female mongoloids which included 9 male and 15 female adults (Tables 1, 2 and 3). These mongoloids are second generation Australians living in Melbourne.

The maximum head breadth, the maximum head length, and the horizontal head circumference have been measured according to the instructions of Martin & Saller (15). All measurements have been recorded to the nearest millimetre.

### Findings and Discussion

#### 1. Maximum head breadth

(Tables 1 and 2). Figure 1 records the means obtained during this investigation, in addition to those of Provis & Ellis (16) and Westropp & Barber (23) for normal children. The data of these workers has been selected because the children they

studied were well nourished and were racially similar to those included in the present study.

*Male mongoloids:* The average maximum head breadth lies within the normal range until the age of about one year after which it increases more slowly than in normal children until the age of five years. Between the ages of five and nine years this measurement increases more rapidly, though somewhat irregularly, than the average for the normal groups so that by the ninth year the average maximum head breadth is less than 1.0 S.D. below the normal average. After this age, however, there is no progressive increase as groups of older mongoloids are considered, whereas a continuing gradual increase is apparent in groups of normal children.

*Female mongoloids:* The average maximum head breadth is equal to that of normal females until the age of 0.7 years but between this age and 4.5 years there is a much less rapid increase in the mongoloid groups, following which the mongoloids increase at a rate similar to that of normal children although somewhat irregularly. One mongoloid had a maximum head breadth markedly in excess of the normal average. These findings should be compared with those of Benda (4, 5) who

<sup>1</sup> This work was supported by a grant from the Mental Health Research Fund.

TABLE 1. *Male mongoloids.*

All measurements are in centimetres.

Chronological age years			Horizontal head circumference		Maximum head breadth		Maximum head length		Breadth length index
Mean	S.D.	Number	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean
0.1	0.03	2	35.8	1.56	9.9	0.07	12.2	0.57	80.9
0.5	0.03	3	39.6	1.63	11.2	0.38	12.8	0.70	87.8
0.7	0.07	6	40.7	1.07	11.8	0.14	13.0	0.57	91.2
1.3	0.25	4	43.5	1.21	12.5	0.55	14.2	0.26	89.0
2.6	0.09	9	45.5	1.33	12.8	0.34	14.8	0.41	86.1
3.0	0.22	3	44.9	1.01	12.7	0.15	14.8	0.36	85.6
3.4	0.15	3	45.3	1.74	13.2	0.90	14.3	0.32	91.9
3.8	0.06	2	45.7	2.26	12.4	0.00	15.8	1.27	80.8
4.7	—	1	44.6	—	13.2	—	14.6	—	90.4
5.3	0.44	6	47.9	1.35	13.6	0.40	15.7	0.65	86.7
6.7	0.47	5	48.1	1.92	13.4	0.71	15.9	0.45	84.5
8.7	0.33	2	49.4	1.18	14.0	0.07	16.2	0.21	86.4
9.5	—	1	49.8	—	14.4	—	16.2	—	88.9
11.4	—	1	51.1	—	14.6	—	16.5	—	88.5
13.5	0.46	2	50.3	1.97	14.3	1.14	16.6	0.30	86.1
14.6	1.1	3	51.2	1.90	14.6	1.00	16.6	0.31	88.3
15.8	0.01	2	48.5	2.20	14.0	—	16.3	1.38	86.5
16.6	—	1	50.5	—	14.6	—	16.4	—	89.0
17.4	0.32	2	52.4	1.41	14.4	0.28	17.4	0.21	83.0

TABLE 2. *Female mongoloids.*

All measurements are in centimetres.

Chronological age years			Horizontal head circumference		Maximum head breadth		Maximum head length		Breadth length index
Mean	S.D.	Number	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean
0.1	—	2	35.7	0.85	9.8	0.21	11.9	0.28	82.0
0.3	0.03	2	37.6	0.42	10.8	0.14	12.1	0.42	89.3
0.5	0.07	3	38.7	1.46	10.9	0.61	12.2	0.48	87.3
0.7	0.17	4	40.7	0.68	11.7	0.28	13.2	0.17	88.3
1.1	0.32	3	43.1	1.56	11.9	0.30	14.1	0.85	84.4
1.5	0.15	8	42.6	1.16	12.2	0.65	13.9	0.72	87.7
2.0	0.21	3	43.0	3.82	12.0	0.72	14.2	0.51	84.7
2.4	0.09	2	43.2	0.49	12.2	0.14	14.5	0.07	84.4
3.5	0.07	2	43.1	4.25	12.4	0.07	14.0	0.43	87.9
5.3	0.37	5	47.3	2.50	13.3	0.64	15.7	0.84	85.0
7.0	0.44	2	47.0	2.83	13.3	0.07	15.6	1.35	85.5
8.4	0.89	2	47.5	1.76	13.3	0.50	15.9	0.64	84.6
9.3	0.34	2	50.1	0.78	13.0	0.14	16.8	0.14	77.4
10.1	—	1	47.0	—	15.8	—	15.6	—	100.3
11.5	0.53	5	48.0	0.53	13.4	0.37	16.1	0.23	85.0
12.6	0.41	5	48.7	1.32	13.6	0.28	16.2	0.81	84.7
13.6	0.34	4	48.1	3.30	13.3	0.93	16.1	1.03	84.9
14.2	0.20	6	49.1	1.58	13.8	0.39	16.5	0.73	84.9
15.3	—	1	51.8	—	14.6	—	16.5	—	84.5
16.1	—	1	51.7	—	13.9	—	16.9	—	84.3
17.5	0.13	3	49.6	0.86	13.9	0.31	16.4	0.15	85.0

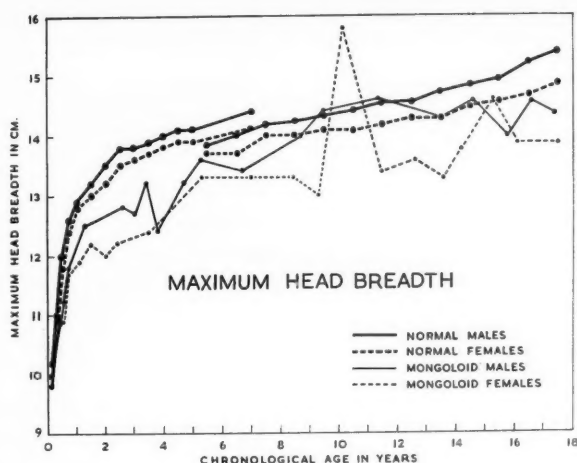


Fig. 1.

recorded a gradual rise in this measurement in his pooled data for male and female mongoloids.

*Male and female mongoloids aged more than eighteen years* (Table 3). The average values for the males are approximately equal to those for normal sixteen-year-old boys while those of the females are approximately equal to the average for normal girls aged thirteen years.

## 2. Maximum head length

(Tables 1 and 2 and Figure 2). During the first few months of life there is little difference between the average values in mongoloid and normal children of both sexes. This difference increases, however, as progressively older groups are considered and reaches a maximum of  $-3.0$  to  $-4.0$  S.D. at about the age of five years. From this age until eight years the average

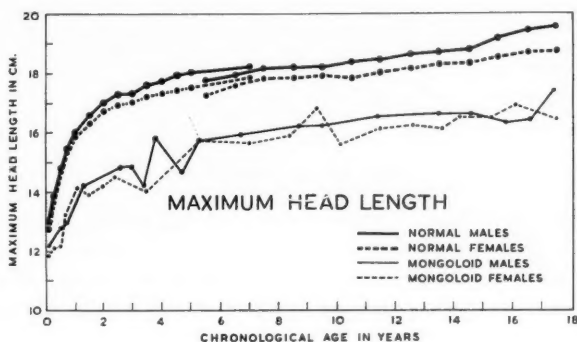


Fig. 2.

TABLE 3. *Head measurements on individual mongoloids aged more than 18 years*

All measurements are in centimetres.

Chronological age years	Horizontal head circumference	Maximum head length	Maximum head breadth	Breadth length index
18.7 M	53.4	17.5	14.6	83.4
20.0 M	52.4	17.9	14.1	78.8
21.5 M	50.1	16.9	13.7	81.1
27.0 M	52.9	17.8	14.6	82.0
27.1 M	53.2	18.2	14.7	80.8
28.3 M	52.9	17.3	14.7	85.0
30.5 M	55.4	19.1	14.7	77.0
33.2 M	53.8	17.9	15.1	84.4
38.0 M	55.0	17.5	14.2	81.1
20.5 F	49.8	16.3	13.4	83.3
21.0 F	53.4	17.6	14.7	83.5
23.2 F	55.8	19.5	14.7	75.4
24.1 F	47.9	15.6	13.4	85.9
26.5 F	50.4	16.7	13.8	82.6
29.3 F	51.6	16.8	14.4	85.7
30.8 F	48.9	16.1	13.8	85.7
31.4 F	47.5	16.0	13.8	86.3
32.0 F	49.1	16.2	13.7	84.6
32.9 F	50.4	16.7	13.6	81.4
33.1 F	51.2	16.9	13.8	81.7
33.5 F	48.6	15.9	13.2	83.0
34.0 F	48.6	16.4	13.7	83.5
34.3 F	48.5	15.6	14.0	89.7
39.5 F	50.9	17.2	13.7	79.7

M = denotes male; F = denotes female.

values for both mongoloid and normal children increase at similar rates. These findings are in agreement with those previously reported.

*Male and female mongoloids aged more than eighteen years:* Few such mongoloids had a maximum head length in excess of the average for normal eight-year-old children (Table 3). However, four males and five females had a maximum head length within the normal range ( $\pm 2$  S.D.) for normal individuals of the same chronological age.

Some workers report an accelerated increase in head length in normal boys at about the age of 15 years (Boas (8); Wissler (24)), but no such change was observed by

Boas & Wissler (9) or by Goldstein (11). Such a change was observed in the data for males at about the age of 17.4 years but because this is not a serial study it might indicate that male mongoloids with head lengths approaching normal values are more likely to survive.

In normal adults the maximum head length is positively correlated with body length (Martin & Saller, 15) and the short stature of mongoloids might partly explain their low maximum head length.

### 3. Breadth-length index

(Tables 1 and 2; Figure 3). For all age groups except one the average breadth-length index is much higher than in normal

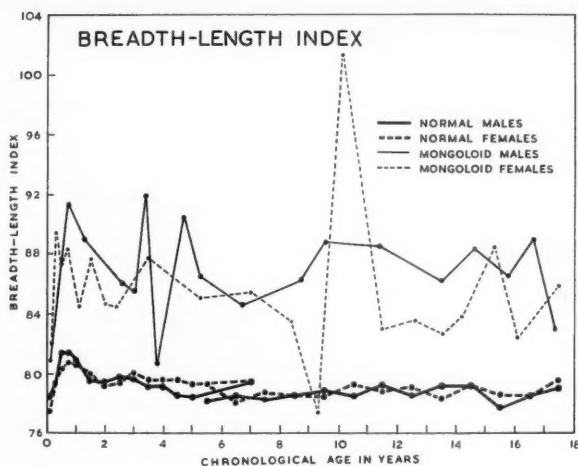


Fig. 3.

children even when due allowance is made for the small length of the mongoloid cranium. The index in both sexes rises in the first few months of life after which there are irregular but not progressive changes. These are similar to the changes reported for normal children (Westropp & Barber, 23). As different age groups of mongoloid children are considered, marked fluctuations in the index are evident which do not obscure the high average index in the mongoloid groups. Almost all these mongoloid children were brachycephalic or hyperbrachycephalic except for six males and ten females who were mesocephalic and one dolichocephalic male. The index was equal to or in excess of 100 in four individuals.

*Male and female mongoloids aged more than eighteen years:* (Table 3). The adult males have a much lower index than the younger males ( $t = 3.37$ ; 18 d.f.) but no such difference is apparent between the groups of females.

It is difficult to determine why the maximum head length shows a greater variation from normal than the maximum head breadth. Howells (13) considers that the growth of the skull tends to be antero-posterior and that the growth of the brain tends to be transverse. A view that the balance between these factors is responsible for the general form of the cranium, would lead us to conclude that in mongolism the rate of brain growth is more nearly normal than the rate of skull growth. Such a simplification of the problem is difficult to justify.

The mongoloid cranium becomes abnormal in size and form during the first four years of life when the brain is growing rapidly and before the time of maximum muscular growth. This suggests that cerebral influences may be responsible for its abnormality. Mongoloid children are more brachycephalic than normal children during periods when neither are able to support their heads and consequently in-



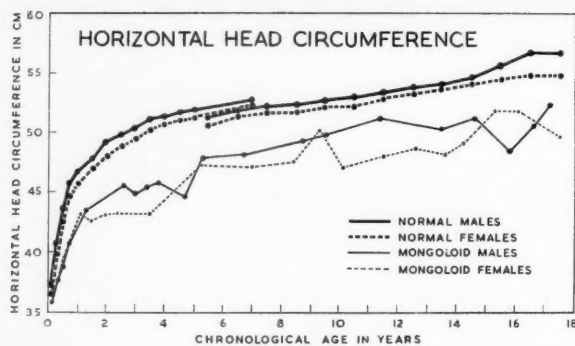


Fig. 4.

creased external occipital pressure cannot be wholly responsible for their brachycephaly.

#### 4. Horizontal head circumference

(Tables 1, 2 and 3 and Figure 4). In both sexes the average circumferences follow normal levels until the age of one year. From then until the fifth year they become progressively lower than those for normal children of corresponding age. After this the rate of increase is approximately the same in the normal and mongoloid groups. Beyond 18 years the majority had a head circumference close to the average for normal children aged 7 to 8 years. These findings are in agreement with those reported by Benda (5). Contrary to the findings of Talbot (20), there were eight males and six females, some of them adults, with a horizontal head circumference within the normal range.

#### Comment

It is clear that there are major variations from normal in the morphology of the mongoloid head. The etiology of these

departures from normal must be considered in relation to the normal mechanism of head growth and the factors influencing them. The observed variations may result from (a) retarded but otherwise normal growth, (b) an imbalance between normal growth processes, or (c) abnormal growth processes. This study has shown that at no stage can the head form be considered equivalent to that of a younger normal child.

There is experimental and clinical evidence that endocrine factors influence the morphology of the cranium. The cranial changes in cretins and in animals following thyroidectomy (Lusted *et al.* (14) among others) resemble those observed in mongoloids except that the head circumference of the latter is small proportional to body size.

Benda (1956) claims that pituitary function is depressed in the mongoloid infant. This view is supported by some experimental evidence (See 1, 2, 3, 10, 19, 21). In addition, hyperpituitarism in children is associated with increased cranial thickness and enlargement of superciliary arches and frontal sinuses (27) which is

the reverse of what is common in mongolism (4, 5, 17, 18). Such findings concerning thyroid and pituitary function are suggestive but they do not provide a complete understanding of mongolism. Other factors e.g. genetic ones cannot be excluded but a consideration of them is beyond the scope of this paper.

### Summary

1. The growth of the head has been investigated in 148 white Australian mongoloids.

2. The maximum head breadth was within the normal range ( $\pm 2$  S.D.) over approximately the first year. In comparison with normal children, the increase for males was then less rapid until the age of five years, more rapid until the age of nine years, after which no continuing increase was observed. In the female group, the changes were similar except for a slight increase throughout the period studied.

3. During first few months of life the

maximum head length was almost normal. It then increased much more slowly than in normal children until about the age of five years after which both groups showed approximately equal rates of increase.

4. The breadth-length index increased rapidly during the first few months of life to become much higher than in normal children, but variable. Most mongoloids are brachycephalic or hyperbrachycephalic, but examples of mesocephaly and dolichocephaly were observed.

5. The horizontal head circumference was normal until about the age of one year, but between this and the fifth year it increased more slowly than in normal children after which the rate of increase was approximately the same.

### Acknowledgements

Our thanks are due to many who have helped during the course of this investigation, particularly the Mental Hygiene Authority of Victoria and their officers and the staff of the Anatomy Department.

### References

1. ASLING, C. W., WALKER, D. G., SIMPSON, M. E., LI, C. H. and EVANS, H. M.: Deaths in rats submitted to hypophysectomy at an extremely early age and the survival effected by growth hormone. *Anat Rec*, 114: 49, 1952.
2. ASLING, C. W., SIMPSON, M. E., MOON, H. D., LI, C. H. and EVANS, H. M.: Growth hormone induced bone and joint changes in the adult rat. The Hypophyseal Growth Hormone, Nature and Actions, p. 154. McGraw-Hill Book Company Inc., New York, 1955.
3. ASLING, C. W. and EVANS, H. M.: Anterior pituitary regulation of skeletal development. The Biochemistry and Physiology of Bone, p. 671. Academic Press Inc., New York, 1956.
4. BENDA, C. E.: Observations on the malformation of the head in mongoloid deficiency. *J Pediat*, 19: 800, 1941.
5. — Mongolism and Cretinism, p. 316. Grune and Stratton Inc., New York, 1949.
6. — Mongolism. A comprehensive review. *Arch Pediat*, 73: 391, 1956.
7. BIEDL, A.: Innere Sekretion; ihre physiologischen Grundlagen und ihre Bedeutung für die Pathologie, p. 480. Urban & Schwarzenberg, Berlin, 1922.
8. BOAS, F.: Changes in bodily form of descendants of immigrants. Reprinted from reports of the U.S. Immigration Commission N.Y., 1912. (Quoted by Wissler, 1930.)
9. BOAS, F. and WISSLER, C.: Statistics of Growth. U.S. Bureau of Education,

- Report of the Commissioner of Education, Washington, 1905. (Quoted by Wissler, 1930.)
10. EVANS, H. M., ASLING, C. W., SIMPSON, M. E. and BECKS, H.: The growth of hypophysectomised female rats following chronic treatment with pure pituitary growth hormone. IV. Skeletal changes; Differences in response from that of intact rats. *Growth*, 13: 191, 1949.
  11. GOLDSTEIN, M. S.: Development of the head in the same individuals. *Hum Biol*, 11: 197, 1939.
  12. HJORTSJÖ, C. H. and LINDEGÅRD, B.: Critical aspects on the use of indices in physical anthropology. *Kungl. Fysiografiska sällskapets i Lund Föreläsningar*, 23: 1, 1953.
  13. HOWELLS, W. W.: The cranial vault; factors of size and shape. *Amer J Phys Anthropol*, 15 (n.s.): 19, 1957.
  14. LUSTED, L. B., PICKERING, D. E., FISHER, D. and SMYTH, F. S.: Growth and metabolism in normal and thyroid-ablated infant Rhesus monkeys (*Macaca Mulatta*). V. Roentgenographic features of skeletal development in normal and thyroid-ablated infant Rhesus monkeys (*Macaca Mulatta*). *Amer J Dis Child*, 86: 426, 1953.
  15. MARTIN, R. and SALLER, K.: *Lehrbuch der Anthropologie*, 5th ed., Gustav Fischer, Stuttgart, 1958.
  16. PROVIS, H. S. and ELLIS, R. W. B.: An anthropometric study of Edinburgh schoolchildren. Part I. Methods, data and assessment of maturity. *Arch Dis Child*, 30: 328, 1955.
  17. ROCHE, A. F., SEWARD, F. S. and SUNDERLAND, S.: Non-metrical observations on cranial radiographs in mongolism. To be published.
  18. ROCHE, A. F., and SUNDERLAND, S.: Post-mortem observations on mongoloid crania. To be published.
  19. SIMPSON, M. E., VAN DYKE, D. C., ASLING, C. W. and EVANS, H. M.: Regeneration of the calvarium in young normal and growth hormone-treated hypophysectomised rats. *Anat Rec*, 115: 615, 1953.
  20. TALBOT, F. B.: Studies in growth. III. Growth of untreated mongolian idiots. *Amer J Dis Child*, 28: 152, 1924.
  21. WALKER, D. G., ASLING, C. W., SIMPSON, M. E., LI, C. H. and EVANS, H. M.: Structural alterations in rats hypophysectomised at six days of age and their correction with growth hormone. *Anat Rec*, 114: 19, 1952.
  22. WEINMANN, J. P., and SICHER, H.: Bone and Bones. Fundamentals of Bone Biology, p. 464. C. V. Mosby Co., St. Louis, 1947.
  23. WESTROPP, C. K. and BARBER, C. R.: Growth of the skull in young children. Part I. Standards of head circumference. *J Neurol Neurosurg Psychiat*, 19: 52, 1956.
  24. WISSLER, C.: Growth of children in Hawaii; based on observations of Louis R. Sullivan. *Memoirs of the Bernice P Bishop Museum*, 11: 257, 1930.

Received May 16, 1960

Accepted July 6, 1960

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## Bone Marrow Aplasia in Childhood Leukaemia

by ERNEST GLÜCK and JON LAMVIK

Hyperplasia of certain elements of the bone marrow and leukaemic infiltrations in the parenchymatous organs form the pathologico-anatomical basis of leukaemia and are the usual findings on post-mortem investigation. Occasional cases in which these findings are absent at post-mortem have been reported previously. Kirshbaum & Preuss (5) found that 14 out of 123 patients dying of leukaemia had a "fatty marrow" at post-mortem. Since the antimetabolite drugs have been used in the treatment of leukaemia, several authors have published reports of bone

marrow hypoplasia or aplasia (3, 7, 8, 9, 11), in some cases as a reversible phenomenon during treatment (8). It has been stated that the hypoplasia of the bone marrow can be a toxic effect of the preparation used (1, 6, 11). We have not been able to find any reports of terminal aplasia of the bone marrow in children with leukaemia.

Between 1950 and 1959 inclusive, 28 children with clinical diagnoses of leukaemia made on the basis of the usual clinical and haematological criteria were autopsied at the Gade Institute. The bone marrow was

TABLE 1. *The haematological and post-mortem findings in six cases of leukaemia with hypoplastic or aplastic marrow.*

Case	Sex	Age, years	Initial haematological findings					Terminal haematological findings			Post-mortem findings	
			Hb %	W.B.C./mm <sup>3</sup> in 1000	Mononuclear cells %	Thrombocytes /mm <sup>3</sup> in 1000	Bone marrow blast cells %	W.B.C./mm <sup>3</sup> in 1000	Mononuclear cells %	Thrombocytes /mm <sup>3</sup> in 1000	Bone marrow activity	Leukaemic infiltrations
1	M	13	39	142	93	0	97	9.7	32	296	Aplastic	Meninges
2	M	12	28	31	99	495	100	0.8	88	9	Hypoplastic	Absent
3	F	2	42	14	83	30	96 <sup>a</sup>	1.6	100	0	Aplastic	Absent
4	F	2	58	12	84	3	70 <sup>a</sup>	2.3	100	0	Hypoplastic	Absent
5	F	14	34	3	73	2	56	2.6	81	0	Aplastic	Absent
6	F	6	76	6	87	34	87	7.1	20	96	Hypoplastic <sup>b</sup>	Absent

<sup>a</sup> Low cellularity.

<sup>b</sup> With megaloblasts.

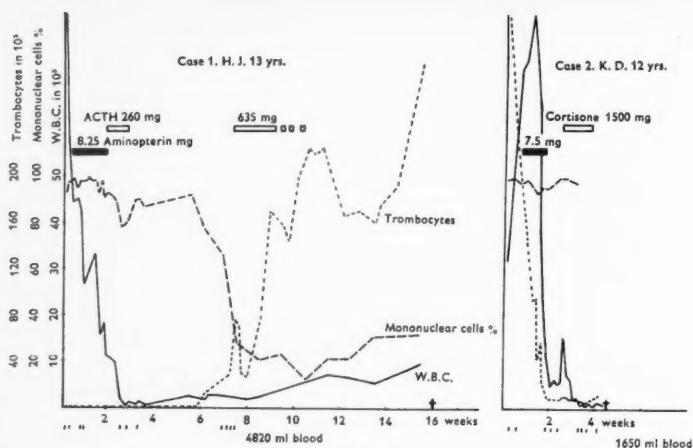


Fig. 1.

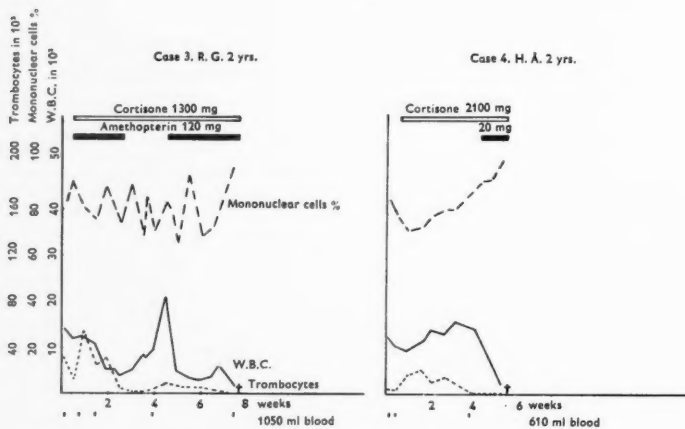


Fig. 2.

Figs. 1 and 2. Cases 1, 2, 3 and 4: Treatment and variation in the haematological findings in the course of disease.

examined microscopically in 21 of these cases, of which six showed hypoplasia or aplasia of the bone marrow. Some of the clinical, haematological and post-mortem findings in the latter group are shown in Table 1 and Figs. 1-3. All the cases were classified as blast-cell leukaemia because the primitive cell forms were so undifferentiated. Erythro- and thrombocytopenia

were present in most of the patients at the time diagnosis was made. Enlargement of the liver and spleen was present in all except Case 5.

All patients with a hypoplastic or aplastic marrow had been treated with folic acid antagonists, whereas 10 of 15 patients with a hyperplastic marrow had been so treated. In the patients with hypoplastic marrow the

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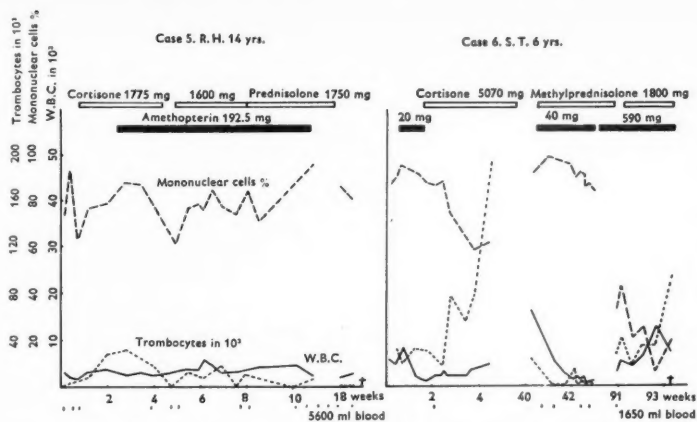


Fig. 3. Cases 5 and 6: Treatment and variation in the haematological findings in the course of disease.

length of the illness varied from 4 weeks to 22 months, reckoning from the onset of symptoms. In Cases 1 and 6 the course of the illness was interrupted by one and two clinical and haematological remissions, respectively. Terminal symptoms from the gastro-intestinal tract, such as stomatitis, vomiting, diarrhoea and persistent diffuse abdominal pain were present in all patients.

Case 1 presented a progressive cachexia. A tendency to bleed was one of the commonest manifestations.

#### Post-mortem investigation

Bone marrow examination from the lumbar vertebrae and the diaphysis of the

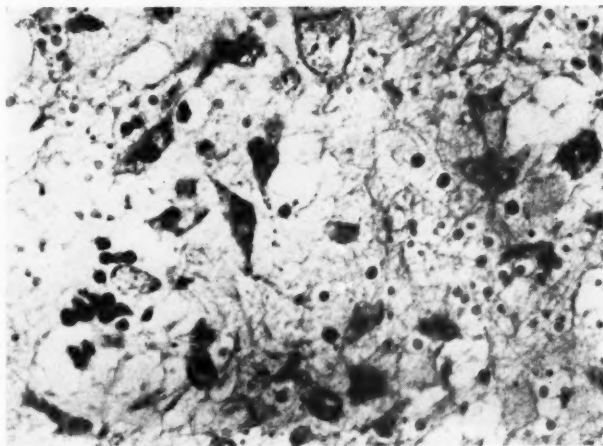


Fig. 4. Case 2: Hypoplastic vertebral marrow with scattered primitive reticulum cells. Left: a group of small mononuclear cells. (H. E.  $\times 460$ .)

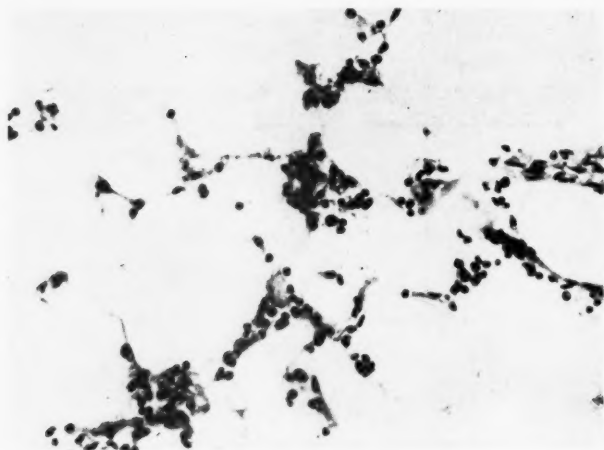


Fig. 5. Case 5: Aplastic vertebral marrow. Mainly fat cells with small groups of lymphocyte-like cells. (H. E.  $\times 350$ .)

femur was performed in addition to the routine macro- and microscopical examination of the organs. In some cases the sternal marrow was also examined. In three children (Cases 1, 3 and 5) both the

spongy vertebral marrow and the marrow from the diaphysis was considered to be aplastic since it consisted mainly of fat with small groups of cells that looked like lymphocytes (Fig. 5). In two children

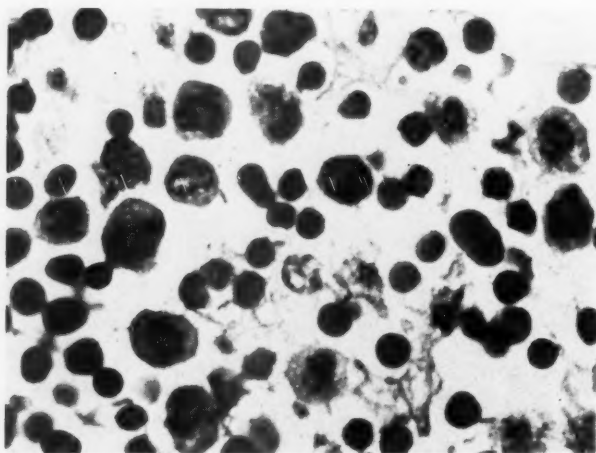
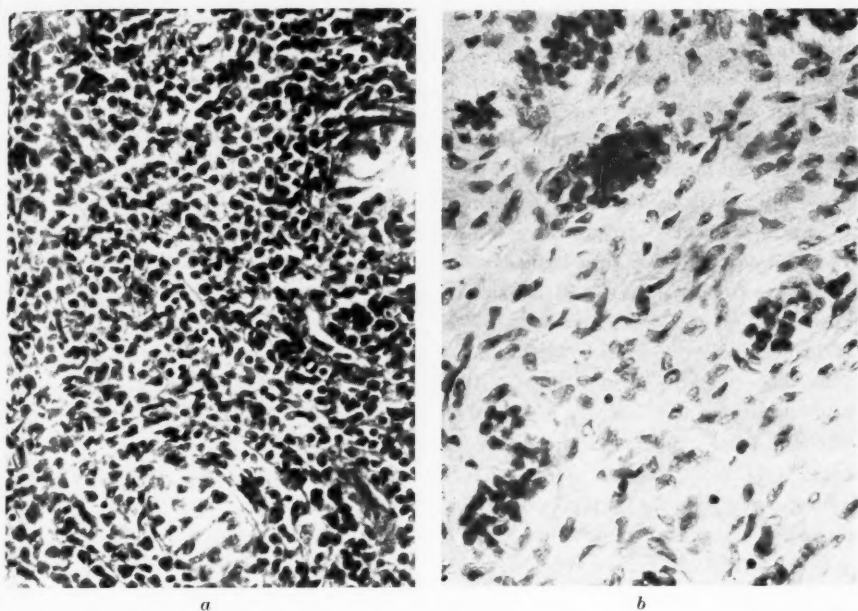


Fig. 6. Case 6: Megaloblastic marrow from the vertebra. Most of these cells are large, irregular megaloblasts. Also present are erythrocytes showing anisocytosis and some small mononuclear cells. (H. E.  $\times 1300$ .)





Figs. 7a and 7b. Case 6: Microphotograph of the testicle. *Left*: The biopsy 1 year before death; *right*: The testicular tissue at post mortem. (H. E.  $\times 350$ .)

(Cases 2 and 4) there were more cells than in the previous group and were therefore termed hypoplastic (Fig. 4). In Case 6 the cellularity of the vertebral marrow was apparently normal, but the picture was dominated by megaloblasts, surrounded by some lymphocyte-like cells (Fig. 6). In this patient compact infiltrations of primitive mononuclear cells were found on testicular biopsy (Fig. 7a). At post-mortem these infiltrations were no longer present and the testes were fibrotic (Fig. 7b). The same patient showed areas in both the ilium and the colon where the mucous membrane was replaced by a cellular granulation tissue, which contained remnants of metaplastic epithelium with some syncytial areas.

Leukaemic infiltrations, most frequently in the liver, spleen, kidney and lymph nodes, were found in all cases with a leukaemic bone marrow. In five of the six cases with hypoplastic or aplastic marrow (Cases 2, 3, 4, 5 and 6) there were no leukaemic infiltrations in the organs examined. One child (Case 1) had an aplastic bone marrow and infiltrates of mononuclear cells in the meninges.

#### Discussion

It is evident from this analysis of 21 cases of clinically proved leukaemia that the process was still active when death occurred in 15 of the cases, judging by the microscopic findings in the bone marrow. In the remaining six cases there was a

distinct lack of leukaemic cell types in the marrow.

Although there is always some concern that the microscopical sections are not representative, the lack of leukaemic infiltrations in the organs, together with the acellular bone marrow, is a strong indication that our bone-marrow samples are representative, and are indicative of the lack of activity in the leukaemic process.

The toxic effect of folic acid antagonists on the bone marrow is first a reduction of megakaryocytes followed by a reduction in the myeloid and erythropoietic elements (11). The myeloid elements are reduced more than the erythropoietic. The normal relationship between these, 4:1, may fall to less than 1:1 (12). Lymphoid tissue seems to be less affected. The commonest clinical manifestations of toxic effect are a tendency to haemorrhages and digestive tract symptoms, including stomatitis, glossitis, vomiting, abdominal pain and diarrhoea. Ulcers may occur in the mucous membranes of the mouth and the intestines (11). Squamous metaplasia of the intestinal and bronchial epithelium has been seen in children treated with folic acid antagonists (13). In the bone marrow one may find an increase in the number of hypersegmented polymorphonuclear cells and giant types of metamyelocytes (12), and megaloblasts may appear after a long period of treatment with aminopterin (2, 7, 12). Leukaemic patients under treatment may have an increase in the differentiated granulocytes in the peripheral blood, in spite of the fact that the patient at the same time shows a reduction in the leucocyte count (8). The adrenocortical steroids and ACTH

which are often used in conjunction with the folic acid antagonists may improve the regeneration of the normal marrow elements (6). As far as we are aware, hypoplasia of the bone marrow has not been reported following hormone treatment alone.

The haematological changes and the post-mortem findings of the aplastic hypoplastic group can be divided into several categories. In two of the patients (Cases 3 and 4) the bone marrow, in spite of a moderate leucocytosis, showed low cellularity from the time the diagnosis was made, that is, before any treatment had been started. Both patients were 2 years of age. One had had radium treatment for a haemangioma and the other was a mongol, both of which have a known relationship to the occurrence of leukaemia. Following the combined treatment with hormones and folic acid antagonists, a drop in the number of white blood cells and thrombocytes occurred but there was no haematological remission. It is reasonable to believe that the process in these patients was aplastic from the start and that the treatment may possibly have furthered this development. It is doubtful whether the folic acid antagonists can give a therapeutic advantage in patients in whom the bone marrow shows an aplastic tendency primarily.

Two patients in this group (Cases 2 and 5) had a very cellular bone marrow initially. Folic acid antagonists did not, however, give the expected haematological remission and the primitive cells remained proportionally very high during the entire illness. In Case 2 there was a considerable fall in the numbers of leucocytes and thrombocytes just after the folic acid

antagonists had been given. In this case there seemed to be a clear relationship between the treatment with folic acid antagonists and the haematological changes. Case 5 had marked thrombocytopenia and leucopenia during the whole course of the illness. The effect of the treatment on the development of the aplasia of the bone marrow was not directly evident. No enlargement of the liver and spleen was ever noticed in this patient.

The last two patients in this group (Cases 1 and 6) had haematological remissions during treatment with folic acid antagonists. Case 1 showed the clinical signs of steady progression of the illness, in spite of normalisation of the haematological findings. The illness was characterized by severe pains in the extremities. A cachectic condition later developed with terminal stupor and coma. In spite of considerable enlargement of the liver and lymph nodes early in the illness there was no enlargement or leukaemic infiltrations at autopsy except for infiltrations in the meninges. Persistent leukaemic infiltration of the central nervous system in spite of haematological remission has been reported recently (4). This apparent resistance to treatment with folic acid antagonists is attributed to lack of penetration by the drug, as it is thought that it is difficult for the drug to pass the blood-brain barrier. The intrathecal administration of folic acid antagonists and steroid and X-ray therapy has been recommended in cases with central nervous system symptoms (4, 10).

The dose of folic acid antagonists was small in Case 1 and was stopped 3 months before death. However, very small doses of folic acid antagonists have been

reported to have a maximal and prolonged effect in cachectic patients who may have a deficiency of folic acid (11). On the other hand, large doses were used in Case 6, 650 mg amethopterin spread over a period of 37 weeks, and the treatment continued right up to the death. In this patient intestinal changes characteristic of aminopterin intoxication were found at autopsy. Further, the bone marrow in both these patients showed a very intense megaloblastic reaction with giant forms, as can be found in folic acid deficiency.

### Summary

Six of 21 children with blast cell leukaemia were found at post-mortem to have a hypoplastic or aplastic bone marrow. Leukaemic infiltrations were found in the organs of all the cases with a hyperplastic marrow, but were present in only one of the cases in the aplastic group and are limited to the meninges. The cause of aplasia of the bone marrow in leukaemia is discussed, particularly with regard to the possible influence of the folic acid antagonists. In two of the cases the bone marrow biopsies were indicative of an aplastic course from the start. The treatment in these cases seems to have reduced the activity of the bone marrow still further. In one case the aplasia developed during prolonged treatment with folic acid antagonists; a causal relationship is, however, uncertain. In the remaining three cases there was a haematological response after treatment with folic acid antagonists. At autopsy changes that could have been due to lack of folic acid were found in the organs.

## References

1. DAMESHEK, W. and GUNZ, F.: Leukemia. Grune & Stratton. New York and London, 1959.
2. HAMILTON, L., PHILIPS, F. S., STERNBERG, S. S., CLARKE, D. A. and HITCHINGS, G. H.: Hematological effects of certain 2,4-diaminopyrimidines, antagonists of folic acid metabolism. *Blood*, 9: 1062, 1954.
3. HAYHOE, F. G. and KOK, A.: Medullary aplasia in chronic myeloid leukaemia during busulphan therapy. *Brit Med J*, 4: 1468, 1957.
4. HOVEID, P.: Akutt myelogen leukemi (promyelocytiske leukemi) med progredierende meningeal og cerebral affeksjon under hematologisk remisjon. *Nord Med*, 62: 1521, 1959.
5. KIRSHBAUM, J. D. and PREUSS, F. S.: Leukemia; clinical and pathologic study of 123 fatal cases in series of 14,400 necropsies. *Arch Int Med*, 71: 777, 1943.
6. PIERCE, M. J.: The acute leukemias of childhood, p. 497. *Pediat Clin N Amer*, May 1957.
7. SCHOENBACH, E. B., COLSKY, J. and GREENSPAN, E. M.: Observations on effects of folic acid antagonists, aminopterin and amethopterin, in patients with advanced neoplasms. *Cancer*, 5: 1201, 1952.
8. SMITH, C. H. and BELL, W. R.: Aminopterin in treatment of leukemia in children; serial aspirations of bone marrow as guide to management and appraisal of treatment. *Amer J Dis Child*, 79: 1031, 1950.
9. STICHNEY, J. M., HAGEDORN, A. B., MILL, S. D. and COOPER, T.: Changes in blood and bone marrow in acute leukemia induced by aminopterin. *J Lab Clin Med*, 33: 1481, 1948.
10. SULLIVAN, M. P.: Intracranial complications of leukemia in children. *Pediatrics*, 20: 757, 1957.
11. TAYLOR, S. G., HASS, G. M., CRUMRINE, J. L. and SLAUGHTER, D. P.: Toxic reactions of 4-amino-pteroylglutamic acid (aminopterin) in patients with far-advanced neoplastic disease. *Cancer*, 3: 493, 1950.
12. THIERSCH, J. B.: Bone-marrow changes in man after treatment with aminopterin, amethopterin, and aminoanfol, with special reference to megaloblastosis and tumor remission. *Cancer*, 2: 877, 1949.
13. WESTON, J. T. and GUIN, G. H.: Epithelial atypias with chemotherapy in 100 acute childhood leukemias. *Cancer*, 8: 179, 1955.

Received May 15, 1960

Accepted August 13, 1960

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## Venous Hypertension in the Newborn Infant Associated with Delayed Clamping of the Umbilical Cord<sup>1</sup>

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In the course of studies of umbilical vein pressure in the newborn infant a temporary elevation of pressure was noted shortly after birth in several subjects. This report describes these observations, and further studies designed to test the hypothesis that this venous hypertension might be caused by rapid infusion of blood from the placenta associated with delayed clamping of the umbilical cord.

### Patient Material

Umbilical vein pressure determinations were obtained on a total of 62 infants during the first four days of life. These infants have been divided into six groups:

*Group A* consists of 24 full-term and 7 premature infants on whom a single determination of umbilical vein pressure was made. Their ages ranged from 5 to 89 hours. None of the subjects in this group had clinical evidence of cardiac or respiratory difficulty. Six of the premature infants were studied prior to the beginning of exchange transfusion for physiological jaundice. The remaining infants in this group were chosen at random. The time at which the umbilical cord was clamped in these infants was not known.

Supported by the Twenty-Five Club, a Woman's Auxiliary of the Elizabeth Steel Magee Hospital.

Infants in Group B through F were all delivered during the day and were considered to be mature. No other factors entered into the selection of these infants. Continuous observations of umbilical venous pressure were made on these infants beginning shortly after the time of delivery and ending, in most cases, after the pressure had stabilized. A single repeat reading was made between the ages of 13 and 30 hours on infants in Groups B, E, and F, with the exception of one infant in Group B who had two repeat readings at 3 and 22 hours. The umbilical cord was clamped within a few seconds of birth on infants in Groups D and F, and after a delay of several minutes on infants in Groups B, C, and E.

*Group B* contains the six infants, all delivered *per vaginam*, on whom the initial studies of umbilical venous pressure at the time of delivery were carried out. These infants, with umbilical cords intact, were placed on a table 30-45 cm below the level of the mothers' perineum, and the umbilical cord was manipulated and punctured almost immediately thereafter. The time of clamping of the umbilical cord varied from 2-6 minutes after delivery in these infants. The umbilical cord was stripped in one case.

*Group C* contains nine full-term infants delivered *per vaginam* in whom a period of about 3 minutes elapsed between the time of delivery and clamping of the umbilical cord. The infants were placed upon a table 30-45 cm below the level of the mothers' perineum,

and were not handled except for gentle aspiration of secretions from the nose and throat; the umbilical cord was not manipulated during this period and care was taken to prevent it's being subjected to undue tension.

*Group D* consists of six full-term infants delivered by the vaginal route whose umbilical cords were clamped within a few seconds of delivery, during which time they were held above the mother's abdomen.

Infants in Groups E and F were delivered by low cervical cesarean section prior to onset of labor.

*Group E* contains five full-term infants who were placed as low as possible on the operative field immediately after delivery. The placenta was then delivered and the infant and the placenta were removed together to a table upon which the infant was placed. The placenta was then held as far above the infant as feasible without traction's being placed on the cord, with the fetal surface of the placenta facing the infant. This position was held for three minutes, or until the cord vein appeared collapsed. In four studies the cord was stripped throughout it's entire length in the direction of the baby.

*Group F* consists of four full-term infants and one premature infant, whose umbilical cords were clamped at the time of delivery.

Estimation of the volume of blood remaining in the placenta following either early or delayed clamping was made on a separate group of 28 infants, all of whom were full-term and delivered by the vaginal route.

### Methods

*Umbilical vein pressure* was measured in mm of normal saline; values were read directly from a simple manometer. The saline contained approximately two units of Heparin® per ml. The infants were studied in the supine position. The mid-axillary line was chosen as point of reference for the manometer as it approximates the level of the right auricle. In the Group B infants the umbilical vein was punctured with a large

needle, the needle withdrawn, and the umbilical vein catheterized through the rent in the vessel. Attempts to obtain pressure readings prior to cessation of the blood flow in the umbilical vessels were successful in but one of the six infants in this group. In infants in Group A and Groups C through F the umbilical vein was incised within 2 cm of the abdomen prior to catheterization and a polyethylene or polyvinyl catheter filled with saline was inserted into the umbilical vein until marked and rapid excursions of pressure occurred immediately with changes in the infant's activity. Withdrawing the catheter 2 or 3 cm from the point at which maximal fluctuations were noted did not modify the venous pressure reading. Measurements obtained at autopsy indicate that the junction of the umbilical vein and ductus venosus is found at a distance of 8-9 cm from the umbilicus in full-term infants. The majority of the venous pressure readings were obtained at depths of insertion (measured from the junction of the umbilicus and the abdominal wall) of 7 cm in the full-term infants, and of 6 cm in the premature infants. Occasional readings taken with the catheter inserted deeper than 8 cm were deleted. Hence it is believed that the values reported were obtained with the tip of the catheter in the recess of the umbilical vein.

The influence of crying and general muscular activity on the infant's umbilical vein pressure introduced the greatest possible source of error in these data. Readings were accepted only when the pressure was constant for at least several seconds, when the fluctuations normally seen with inspirations were noted, and when the observers judged the infants to be maximally relaxed. Such an evaluation is necessarily somewhat subjective.

All infants were given intramuscular penicillin and dihydrostreptomycin for the three days following the umbilical vein catheterization.

### Results

The mean umbilical vein pressure of 31 infants from 5 to 89 hours of age

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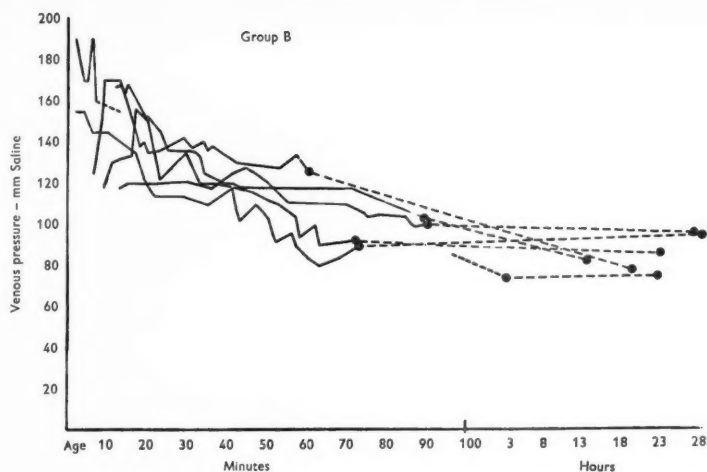


Fig. 1. Umbilical vein pressure patterns of infants with delayed clamping of the umbilical cord (Group B).

The umbilical cord was manipulated and punctured immediately after birth in these infants. Solid lines connect values obtained on initial insertion of the catheter. Dotted lines connect the last of these values and the single repeat reading or readings.

(Group A) was  $92.2 \text{ mm}$  of normal saline  $\pm 12.8 \text{ mm}$  (S.D.). Age and birth weight did not influence the level of umbilical vein pressure. The highest pressure was  $112 \text{ mm}$ .

Observations on the initial series of infants studied from birth (Group B) are shown in Figure 1. Each of these six infants had umbilical vein pressures above the range defined by the Group A infants at an older age; five of the six had pressures in excess of  $150 \text{ mm}$  at some time during the first twenty minutes of life. The highest pressure was recorded in the only subject in whom the umbilical vein was successfully catheterized with the cord intact and pulsating vigorously. This infant's umbilical vein pressure was  $190 \text{ mm}$  at two minutes of age, dropped to  $170 \text{ mm}$  with the onset of respirations one minute

later, and then rose transiently to  $190 \text{ mm}$  following vigorous stripping of the umbilical cord. Clamping of the cord, immediately after the final stripping, was followed by a drop in pressure to  $160 \text{ mm}$  within one minute.

Continuous observation of umbilical vein pressure was made on four of these infants until their pressure had stabilized within the Group A range. Stabilization took place within 30–90 minutes of age. Continuous recordings were abandoned while venous pressures were still elevated in two infants at 8 and 60 minutes of age. Repeat determinations on these subjects, at 3 and 19 hours respectively, were within the Group A range. Three of the Group B infants breathed spontaneously at birth, the remaining three from 3–6 minutes after delivery. The time of onset



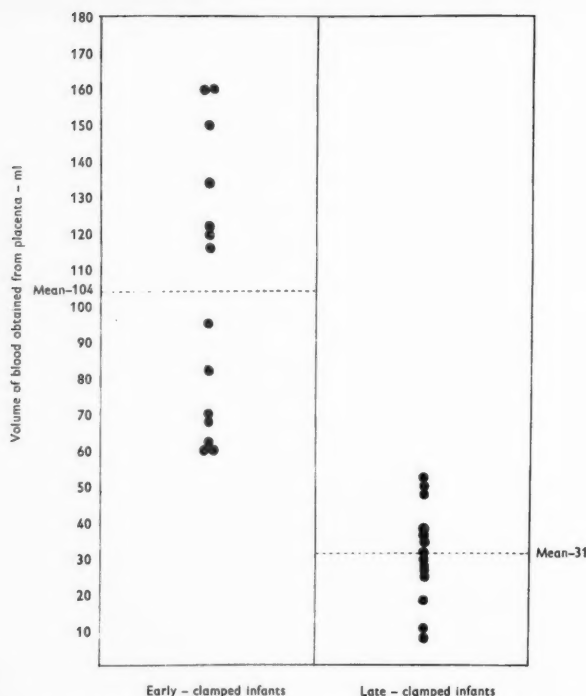


Fig. 2. Volume of blood obtained from the placenta in 14 early- and 14 late-clamped infants.

of respiration did not seem to bear any clear relationship to the infant's venous pressure pattern.

An estimation was then made of the volume of blood transferred from placenta to full-term infant during a three minute delay in clamping of the cord. It was assumed that the difference between the mean volumes of blood obtained from the placentas of series of early- and of late-clamped infants would approximate the mean volume of the placental transfusion. The mean volumes of blood obtained from the placentas of 14 infants with cords clamped at birth, and of 14 infants with cords clamped three minutes after birth,

were  $104 \text{ ml} \pm 37.8 \text{ ml}$  (S.D.) and  $31 \text{ ml} \pm 14.1 \text{ ml}$  respectively (Figure 2). The difference between these means, 73 ml, is significant ( $P < 0.001$ ). These early and late-clamped infants are comparable as to route of delivery (all *per vaginam*), mean birth weight (early: 3330 g; late: 3190 g) and range of birth weight (early: 2700 to 3912 g; late: 2495 to 3865 g).

It seemed likely, therefore, that the infants in Group B received relatively large infusions of placental blood, yet it occurred to us that the vigorous manipulation and puncturing of the cord that started immediately after birth might modify blood flow through the placental

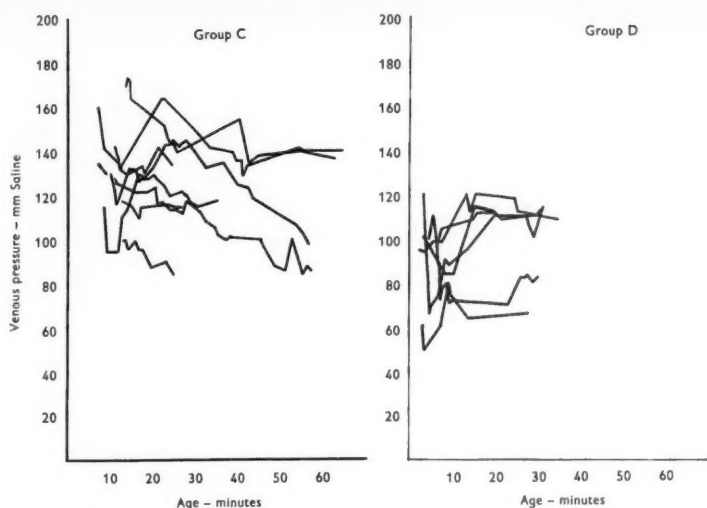


Fig. 3. Umbilical vein pressure patterns of early-clamped infants (Group D), and late-clamped infants (Group C) delivered vaginally. Group C differs from Group B in that the umbilical cords of the Group C infants were not handled prior to clamping.

vessels. Care was taken in the remaining studies to avoid touching or stretching the cord prior to its being clamped. Moreover, an attempt was made from this time on to place the vaginally delivered infants at a uniform distance below the level of the mother's perineum; the studies of Duckman *et al.* (9) and Gunther (11) have emphasized that positioning the infant below the mother facilitates passage of blood from placenta to infant.

Figure 3 allows comparison of the pressure patterns obtained on early-clamped (Group D) and intentionally late-clamped (Group C) infants delivered *per vaginam*. The range of pressures recorded in the early-clamped infants is markedly lower than the range of values observed in the late-clamped group. The highest pressure recorded for the early-clamped infants was 120 mm; seven of the nine late-clamped

infants had venous pressures repeatedly in excess of 120 mm during at least the first twenty minutes of life.

The study of infants delivered by repeat cesarean section prior to onset of labor was undertaken to exclude the possible influence of various factors associated with vaginal delivery on the infant's venous pressure. The infants in these groups breathed within a few seconds to 55 seconds after birth and were all in good condition at delivery. Two infants in the early-clamped group (F) had minimal respiratory distress from birth; both were breathing normally by the age of 30 minutes. Figure 4 shows that the early-clamped (Group F) infants' venous pressures fall within the range of the Group A infants' pressures. Four of the five infants in the late-clamped group (E) had pressures higher than the maximum venous pres-

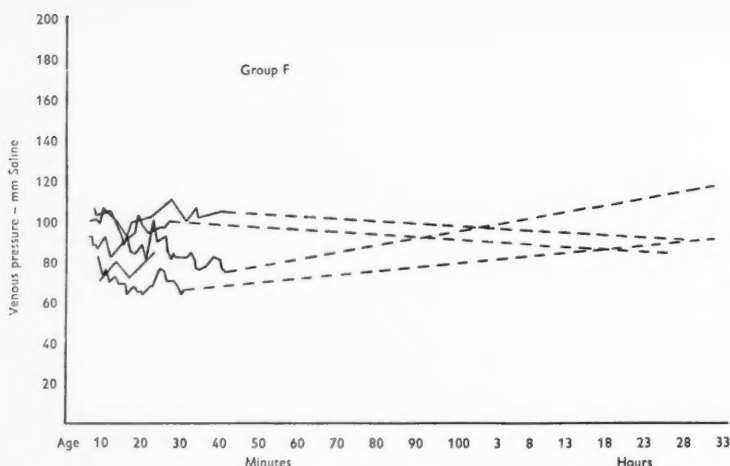


Fig. 4. Umbilical vein pressure patterns of early-clamped infants delivered by cesarean section.

sure of 110 mm recorded for Group F infants (Figure 5). Repeat single determinations were made on nine of these infants between 25 and 31 hours of age; all were within the normal range.

### Discussion

The umbilical vein pressures of the Group A infants are within the range of the peripheral venous pressure reported for normal adults by Winsor & Burch (27), and for normal children reported by Burch (5) and Jacques (14), and are somewhat above the pressure reported by Lambert (16) for normal children. Bonham Carter, Bound & Smellie (3) found the mean umbilical vein pressure of normal infants during the first day of life to be  $26 \text{ mm} \pm 12 \text{ mm}$  (S.D.), using the sternal angle as point of reference for the manometer. We have found the mean difference between the mid-axillary line and the sternal angle to be 37 mm in full term infants, and

somewhat less in prematures. Addition of this difference to the mean value reported by Bonham Carter *et al.*, gives an adjusted mean of 63 mm. The difference between their adjusted mean value and our mean of  $92.2 \text{ mm} \pm 12.8 \text{ mm}$  is significant ( $t = 9.4$ ) and unexplained. Our finding that venous pressure is not influenced by birth weight or by age is in accord with the observations of Bonham Carter *et al.*

The influence of early *versus* delayed clamping of the umbilical cord on the infant's venous pressure during the first minutes of life seems clear from our data. The eleven infants whose cords were clamped within a few seconds after delivery had umbilical vein pressure within or slightly above the range of pressure of the older Group A subjects. The maximum pressure seen in the early-clamped groups was 120 mm; this value was recorded on two of the eleven infants. Elevation of venous pressure of the order of that noted in the initial series studied from birth

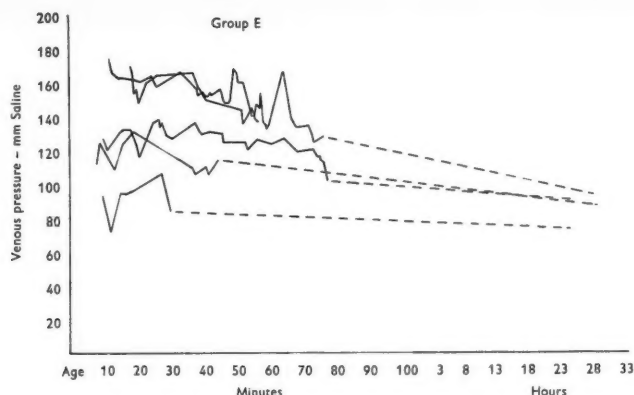


Fig. 5. Umbilical vein pressure patterns of late-clamped infants delivered by cesarean section.

(Group B) was recorded for most of the intentionally late-clamped infants; 11 of the 14 infants in Groups D and F had umbilical vein pressures higher than 120 mm. We have observed umbilical vein pressure in excess of 120 mm after the first hour or so of life only in infants with the clinical findings of congestive heart failure (25). Parkinson (20) has reported umbilical vein pressures of 80–98 mm H<sub>2</sub>O during the first four minutes of life in a series of 14 normal infants whose cords were not stripped, and a pressure of 180 mm H<sub>2</sub>O at the age of 2–3 minutes on one infant whose umbilical cord had been stripped.

The only consistent difference in management between the Group C and D subjects, and between the Group E and F subjects was the time following delivery at which the umbilical cord was clamped. Delay in clamping of the cord, under the conditions previously outlined, effects a transfer of an average of 73 ml of blood from the placenta to the fetal circulation. Haselhorst & Allmeling (12), who recorded

weight gain for successive minutes following delivery in 120 infants with intact umbilical cords, found an average gain of 70 g at the end of three minutes.

The extent of the average placental transfusion is best understood when expressed in terms of percent of the infant's blood volume prior to transfusion. Assuming the initial blood volume to be about 10% of the infant's body weight, then an infant weighing 3500 g would receive a 20% increase in blood volume during the first three minutes of life. According to the data of Haselhorst & Allmeling (12), increases of about 14, 5, and 1% of the infant's initial blood volume take place during the first, second, and third minutes after delivery, respectively. Cutting *et al.* (6) using 1% saline or 5% dextrose solution, and Landis and his co-workers (17) using Ringer's solution have most closely approximated this rate of infusion under experimental conditions. They injected animals with these solutions at a rate sufficient to increase their blood volume from 3–7% per minute. These animals

showed an abrupt rise in venous pressure beginning a few minutes after the start of the infusion. The fastest rate of intravenous injection in human experiments was reported by Sharpey-Schafer & Wallace (23) who injected either normal saline or serum at a rate sufficient to increase blood volume 2–2.5 % per minute. They observed a rise in venous pressure within 2–3 minutes from the start of the infusion. In view of these studies, it is not surprising that most of the late-clamped infants showed an elevated venous pressure shortly after birth.

The experimental data of Henry, Gauer & Sieker (13) on men indicate that change in central venous pressure is directly proportional to change of blood volume induced by bleeding or transfusion. Our experience with the late-clamped infants delivered by cesarean section suggests that a correlation may exist between the volume of blood received by the infant and elevation of his venous pressure. The umbilical cord was not stripped in one infant in Group F, and there was no visible evidence of decompression of cord and placental veins during the time the placenta was held above the infant; his venous pressure was within the normal range. Two irresolute strippings of the cord were done on another infant in this group, his venous pressure was only slightly elevated. Manual compression of the placenta and vigorous stripping of the cord were carried out on the three other infants in this group until the upper portion of the umbilical vein appeared collapsed; all three showed marked elevation of umbilical vein pressure.

The studies of Gauer & Henry (10), Sharpey-Schafer & Wallace (23), and

Warren *et al.* (26) indicate that the return of elevated venous pressure to normal following rapid infusion under experimental conditions is accomplished, in part at least, by reduction of plasma volume. DeMarsh, Windle & Alt (8) and Mollison & Cutbush (18) have shown that hemoconcentration takes place within the first several hours of life in late-clamped infants. Rychek, Tyłka & Taylor (22) found that serial hematocrits taken on late-clamped infants during the first hours of life show, in general, a reciprocal relationship to the venous pressure patterns presented in this report, although marked variation in rate of hematocrit adjustment was noted, while the hematocrit of early-clamped infants changes very little over this period of time. Different rates of adjustment of plasma volume could partially account for the observed differences in rates of decline of venous pressure in late-clamped infants with venous hypertension.

A rapid transfusion of sufficient volume to elevate venous pressure might influence the clinical course of the newborn infant. Budin (4) and Reynolds (21) advocate delayed clamping of the umbilical cord on the thesis that the infused blood benefits the infant by filling his expanding pulmonary vascular bed. Bonham Carter (2) suggests that an increase in cardiac output secondary to the placental transfusion might be effective in initiating expansion of alveoli in the newborn infant. He accepts the evidence advanced by Jäykkä (15) which is purported to show that erection of the pulmonary capillary plexus secondary to increased pulmonary blood flow is an important factor in initiating expansion of the lungs. This seems unlikely, however, as Dawes *et al.* (7) have

shown that the initial inspiration preceeds increase in pulmonary blood flow in fetal lambs. Avery, Frank & Gribetz (1) have demonstrated in experiments on excised lungs that the effect of vascular congestion might facilitate expansion to a limited degree, but was not of sufficient magnitude to initiate it. Moreover, it is not certain that increasing right ventricular output in the apneic infant will increase pulmonary blood flow; all of the increase might traverse the ductus arteriosus. It is possible, as suggested by Bonham Carter (2), that an increase in back pressure from the left auricle on the pulmonary capillaries secondary to the placental transfusion might lower the inspiratory force needed for initial expansion of the lungs.

Temporary elevation of venous pressure secondary to the placental transfusion might be harmful to the newborn infant in some circumstances. Elevation of capillary pressure would increase the possibility of significant hemorrhage into organs such as the brain, the lungs and the adrenal glands which may be traumatized during delivery. Since capillary fragility is greater in the premature than in the mature infant, according to the data of Oehme & Haberland (19) and Ylppö (28), increase in capillary pressure at birth might be especially harmful to the premature infant.

Warren *et al.* (26) have shown that rapid intravenous infusions elevate pulmonary capillary pressure in proportion to the increase in general blood volume in the normal human adult. The relatively large volume of the placental transfusion might increase pulmonary capillary pressure to the point of producing edema. As alveolar edema probably preceeds the ac-

tual pulmonary hyaline membrane it seems reasonable to consider the possible connection between certain cases of the neonatal respiratory distress syndrome and the placental transfusion. This suggestion is based on the assumption that increasing right ventricular output by rapid blood transfusion will increase pulmonary blood flow and pulmonary capillary pressure in the newborn baby. This may not be true, as functional patency of the ductus arteriosus may permit unequal distribution of the increase in blood volume between the systemic and pulmonary circuits.

Speculation will not decide whether the placental transfusion causes significant benefit or harm to the newborn baby. Observations from this clinic on the influence of early *versus* late clamping of the umbilical cord on the clinical course of the newborn infant will be published elsewhere (24).

### Summary

The mean umbilical vein pressure of 31 normal infants 5 to 89 hours of age was 92.2 mm of normal saline  $\pm$  12.8 mm (S.D.). Venous pressure was not influenced by age or birth weight.

Continuous recordings of umbilical vein pressure were made on 11 infants whose umbilical cords were clamped at the time of delivery, and on 20 infants whose cords were clamped several minutes after delivery. The highest pressure recorded in the early-clamped group was 120 mm; 2 of the 11 infants had this pressure. Seventeen of the 20 late-clamped infants had levels of venous pressure repeatedly in excess of 120 mm during the first 30-60 minutes of life with return to the normal range at varying rates.

Possible effects of a placental transfusion sufficiently large to raise venous pressure on the clinical status of the newborn infant have been discussed briefly.

### Acknowledgement

We greatly appreciate the encouragement and guidance offered by Dr. Edmund R.

McCluskey and Dr. Robert Klein during the course of this study. We are also indebted to the Obstetrical Staff of the Elizabeth Steel Magee Hospital, and to the Nursing Staffs of the Delivery Room, Operating Room, and Newborn Nurseries for their active cooperation in obtaining the data presented in this report.

Mrs. Lisl Rosa kindly translated most of the papers from the German literature.

### References

1. AVERY, MARY ELLEN, FRANK, N. R. and GRIBETZ, I.: The inflationary force produced by pulmonary vascular distention in excised lungs. The possible relation of this force to that needed to inflate the lungs at birth. *J Clin Invest*, 38: 453, 1959.
2. BONHAM CARTER, R. E.: The architectural function of pulmonary capillaries. *Lancet*, 272: 1292, 1957.
3. BONHAM CARTER, R. E., BOUND, J. P. and SMELLIE, JEAN M.: Mean venous pressure in the first hours of life. *Lancet*, 271: 1320, 1956.
4. BUDIN, P.: A quel moment doit on practiquer la ligature du cordon ombilical? *Progr Med (Par)*, 3: 750, 1875, and 4: 2, 1876.
5. BURCH, G. E.: A Primer of Venous Pressure. Philadelphia. Lea and Febiger, p. 107, 1950.
6. CUTTING, R. A., LARSON, P. S. and LANDS, A. M.: Cause of death resulting from massive infusions of isotonic solutions. An experimental study. *Arch Surg*, 38: 599, 1939.
7. DAWES, G. S., MOTT, J. C., WIDDICOMBE, J. G. and WYATT, D. G.: Changes in the lungs of the newborn lam. *J Physiol*, 121: 141, 1953.
8. DEMARSH, Q. B., WINDLE, W. F. and ALT, H. L.: Blood volume of newborn infant in relation to early and late clamping of umbilical cord. *Amer J Dis Child*, 63: 1123, 1942.
9. DUCKMAN, S., MERK, H., LEHMANN, W. X. and REGAN, ELIZABETH: The importance of gravity in delayed ligation of the umbilical cord. *Amer J Obstet Gynec*, 66: 1214, 1953.
10. GAUER, O. H. and HENRY, J. P.: Beitrag zur Homöostase des extraarteriellen Kreislaufs. Volumenregulation als unabhängiger physiologischer Parameter. *Klin Wschr*, 34: 356, 1956.
11. GUNTHER, M.: The transfer of blood between baby and placenta in the minutes after birth. *Lancet*, 272: 1277, 1957.
12. HASELHORST, G. and ALMELLING, A.: Die Gewichtszunahme von Neugeborenen infolge postnataler Transfusion. *Z Geburtsh Gynäk*, 98: 103, 1930.
13. HENRY, J. P., GAUER, O. H. and SIEKER, H. O.: The effect of moderate changes in blood volume on left and right atrial pressures. *Circulat Res*, 4: 91, 1956.
14. JACQUES, L. H.: Venous pressure in children. *J Iowa Med Soc*, 32: 294, 1942.
15. JÄYKKÄ, S.: Capillary erection and lung expansion. An experimental study. *Acta Paediat*, 46, Supplement 112, 1957.
16. LAMBERT, J. P.: Venous pressure in children. *Amer J Dis Child*, 52: 1088, 1936.
17. LANDIS, E. M., BROWN, E., FAUTEUX, M. and WISE, C.: Central venous pressure in relation to cardiac "competence", blood volume and exercise. *J Clin Invest*, 25: 237, 1946.
18. MOLLISON, P. L. and CUTBUSH, MARIE: Hemolytic disease of the newborn: Criteria of severity. *Brit Med J*, 1: 123, 1949.
19. OEHME, J. and HABERLAND, R.: Die Kapillarresistenz bei Kindern und älteren Erwachsenen. *Ärztl Wschr*, 12: 673, 1957.
20. PARKINSON, R. P.: A study of venous pressure in the newborn infant. *J Pediat*, 50: 174, 1957.
21. REYNOLDS, S. R. M.: Circulatory adaptations to birth and their clinical implications. *Amer J Obstet Gynec*, 70: 148, 1955.
22. RYCHECK, R. R., TYLKA, ELEANOR, V. and TAYLOR, P. M.: Unpublished observations.
23. SHARPEY-SCHAFER, E. P. and WALLACE, JOAN: Circulatory overloading following rapid intravenous injections. *Brit Med J*, 2: 305, 1942.



24. TAYLOR, P. M., BRIGHT, NANCY H. and BIRCHARD, EDNA L.: The effect of immediate versus delayed clamping of the umbilical cord on mortality and respiratory rates of the newborn infant. In preparation.
25. TAYLOR, P. M., WOLFSON, J. H., BRIGHT, NANCY H., BIRCHARD, EDNA and EGAN, T. J.: Umbilical vein pressure in congestive heart failure in the newborn infant. In preparation.
26. WARREN, J. F., BRANNON, E. S., WEENS, H. S. and STEAD, E. A., Jr: Effect of

increasing the blood volume and right atrial pressure on the circulation of normal subject by intravenous infusions. *Amer J Med*, 4: 193, 1948.

27. WINSOR, T. and BURCH, G. E.: Use of the phlebomanometer: Normal venous pressure values and a study of certain clinical aspects of venous hypertension in man. *Amer Heart J*, 31: 387, 1946.
28. YLPPÖ, A.: Zum Entstehungsmechanismus der Blutungen bei Frühgeburten und Neugeborenen. *Z Kinderheilk*, 38: 32, 1924.

Received May 5, 1960  
Accepted July 13, 1960

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## Sleep Patterns in Infancy

### A Study of One Infant from Birth to Eight Months of Age

by ARTHUR H. PARMELEE, Jr

Sleep and wakefulness are perhaps the most dramatic contrasts in daily human behavior that everyone experiences and observes. Sleep or its opposite, wakefulness, are global complex patterns of behavior. Detailed studies of both states and the transition from one state into the other will give valuable information about the functions of the nervous system as well as other physiological functions of the body.

The most comprehensive review of studies on sleep is that of Kleitman (14), but this only covers the period up to 1939. Subsequently, there have been many studies, particularly relative to the neurophysiology of arousal. These are reviewed in the reports of the Macy Conferences on Consciousness (1), the report of the Quebec Conference on Consciousness (2), and a recent book entitled "The Waking Brain" by Magoun (16).

Studies of the evolution of patterns of behavior in the maturing organism give us a perspective for the study of the complex behavior patterns of the mature organism. We are able to follow the integration of the less complex primitive behavior patterns of the immature organism into the more complex patterns of the mature organism. For man this means observing the evolution of behavior from

infancy to adulthood. Certainly the study of the evolution of sleep and wakefulness patterns of the infant and child may be expected to contribute to our understanding of these processes.

The most recent comprehensive reviews of sleep in infants and children are those of Hellbrügge, Lange & Rutenfranz (10), and Debré & Doumic (5). Gesell & Amatruda (7) have studied sleep in the premature and full term infant. Bühler & Hetzer (4), Kleitman & Engelmann (15), and Moore & Ucko (17), among others, have studied sleep in infancy using samples ranging from 19 to 200 infants. There are, in addition, several detailed reports of the evolution of sleep patterns in just one or a very few infants (7-9, 15, 18, 21).

In general all of these studies indicated that the newborn or very young infant has frequent short sleep periods, and as the infant matures the short sleep periods consolidate into fewer and longer sleep periods and the longest tend to occur more frequently at night. The wakeful periods also consolidate and tend to concentrate in the daytime. Most parents and some doctors have the impression that infants several months old sleep much less than newborn or very young infants. Kleit-

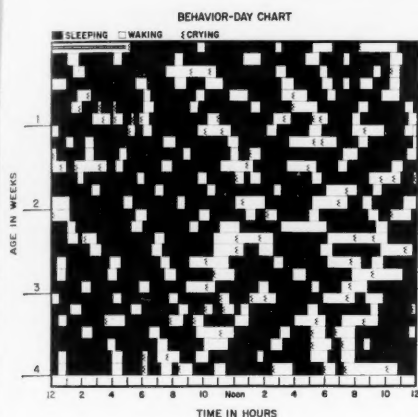


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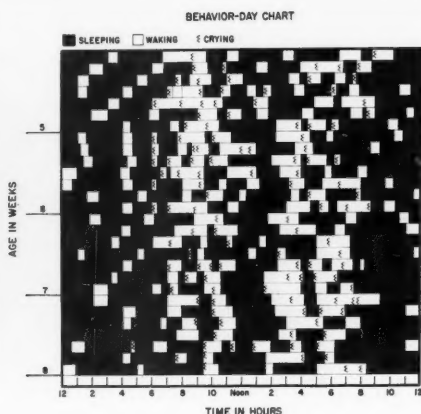


Fig. 2.

man & Engelmann (15), in their very careful study, emphasize the fact that the change in total amount of sleep in 24 hours is not very great, the average total sleep being 14.8 hours at 3 weeks and 13.7 hours at 26 weeks. The major change is in the distribution of the sleep and wakefulness periods.

The material to be presented in this article concerns the behavior day records of one male child from birth to 35 weeks of age. It seems justified to present this single case report since complete daily records of sleep and waking of a baby on a completely free schedule relative to eating, sleeping and waking, conscientiously kept by a mother for 35 weeks, are not readily obtained. Cases documented in such a manner can illustrate the general trends noted in the larger studies and at the same time emphasize individual variation.

#### *Subject of the Observations*

This male child was born after 38 weeks of gestation with a birth weight of 2585 g,

height 47 cm, head circumference 32 cm. He cried immediately after birth and no physical or neurological abnormalities were noted in the neonatal period. His mother's pregnancy had been normal. The labor lasted five hours and the delivery was that of a normal vertex presentation. Nitrous oxide was given to the mother at the time of delivery. She received no medication during labor.

He was fed on a completely self demand schedule. The self demand schedule extended to his sleeping in that he was never intentionally awakened from sleep and every effort was made to allow him to go to sleep when he desired. This started within the hour after his birth since he was in a rooming-in unit with his mother. After the first eight days in this unit in the hospital, he slept in his own room which was a large converted closet attached to the parent's room. The large door to this room was left open and there was no intervening corridor. His movements could be heard but he could not be seen from the parents' room. He received primarily breast feedings with occasional supplemental cow's milk feedings until 10 weeks of age. From then on he received only cow's milk. Purees were started in small amounts at 15 weeks of age.

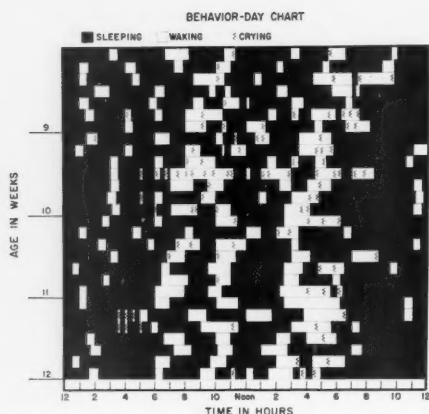


Fig. 3.

Physically and mentally he has progressed normally to the present. He is now twelve years of age.

### Method

The mother of the subject recorded the infant's daily activities on Behavior Day Charts as described by Gesell & Ilg (9). These charts have 24 vertical lines one-half inch apart for the 24 hours of a day, and horizontal lines for recording each day's activities at the proper time interval. These horizontal lines are  $\frac{3}{8}$  inches apart, leaving ample room for recording. There are 28 lines to each chart, thus one chart covers a four week period. In the wide margins of each chart is space for special notes relative to the growth of the child, special occurrences of the day or new developmental achievements, and so forth.

For this subject the mother made her daily recordings with colored pencils. Sleep was recorded as blue, awake and quiet maroon, crying red, breast feeding orange, formula feeding yellow, and water feeding violet. Since each interval between the vertical lines representing one hour of time measured one-half inch, the duration of sleep and wakefulness periods could be recorded to the quarter hour with some accuracy.

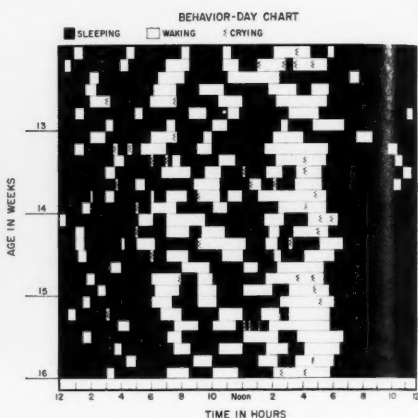


Fig. 4.

For the purposes of illustrating this article the Behavior Day Charts were reconstructed in black and white. In reproducing them in black and white only the sleep (black) and wakefulness (white) were indicated. Crying, indicated by a vertical zigzag line, was only recorded during the first 16 weeks. The reproductions successfully illustrate the patterns of sleep.

The numerical data concerning the duration of total amount of sleep and wakefulness each day, frequency of wakeful periods and longest sleep and wakeful periods, were derived from the original more detailed charts. The comments relative to daily events and developmental progress were obtained from the mother's notes made daily on the original charts.

For the numerical values of duration of sleep and wakefulness the first week of each chart was analyzed. For the first week of life the total sleep and wakefulness of the first day were not included because it was an incomplete day. The longest sleep period of any day included the overlap into the early morning of the next day, since the intention was to determine the ability of the infant to sustain sleep, and after the first weeks this period generally occurred at night and extended over midnight.

TABLE 1. *An analysis of the infant's sleep during the first week recorded on each Behavior Day Chart, including the first and last weeks of the total record.*

(Times are given in hours.)

	(1)		(2)	(3)
	Total sleep per day Av. for week	Range	Av. daily longest sleep per week	Longest sleep period of the week
1st week	17.8	(16.5-20)	4.4	5.5
5th week	15.4	(14-16.5)	4.9	5.0
9th week	18.2	(15.5-20)	5.3	8.5
13th week	17.8	(16.5-19.5)	7.1	8.5
17th week	16.9	(16-18.5)	8.6	9.5
21st week	16.3	(15-17)	10.0	12.0
25th week	15.5	(13.5-17)	11.2	12.0
29th week	16.3	(16-17.5)	11.7	13.0
33rd week	14.4	(13-16)	10.7	12.5
35th week	14.3	(13-15.5)	7.9 <sup>a</sup>	10.0

<sup>a</sup> Mother's notes state that baby was teething at this time and on Behavior Day Chart 9 it can be seen that there is a recurrence of awakening at night.

#### *Discussion of the Behavior Day Charts*

The numerical data presented in Tables 1 and 2 will be discussed first and then the Behavior Day Charts 1 through 9.

In the 1st, 9th and 13th weeks<sup>1</sup> the total amount of sleep per day remained approximately the same. The 5th week was an exception and this will be discussed later. The total amount of sleep very gradually decreased in the succeeding weeks and in the 35th week it was only 3½ hours less than in the 1st week (Table 1, Column 1). The average duration of the longest sleep period per day increased rather rapidly with the most pronounced jumps between the 9th and 13th weeks and between the 17th and 21st weeks. The longest sleep period of each week

followed the same general trend (Table 1, Columns 2 and 3).

This boy, therefore, by the 21st week, had developed the ability to sustain his sleep for periods of a little over twice the duration of those in the first week of life. After the 21st there was not much change.

The total amount of wakefulness was of course the inverse of the total amount of sleep. This gradually increased in duration proportional to the decrease in total amount of sleep (Table 1, Column 1).

The average longest wakeful period for each week studied was slower in lengthening than was the average longest sleep period. By the 21st week the average longest wakeful period was 2.5 hours which was only 1.3 times that in the first week. Only in the 33rd week did this jump to 4 hours which was twice that in the first week (Table 2, Column 2). Nevertheless, there was a steady increase in the average

<sup>1</sup> The numbers of the weeks refer to the seven days preceding the corresponding numbers on the Behavior Day Charts. Thus the first week refers to the seven days from birth to 1 and the 9th week to the seven days between the numbers 8 and 9, and so forth.

TABLE 2. *An analysis of the infant's wakefulness during the first week recorded on each Behavior Day Chart, including the first and last weeks recorded.*

(Times are given in hours.)

	(1)		(2)	(3)	(4)	
	Total wakefulness per day		Av. of daily longest wakeful period per week	Longest wakeful period in week	Frequency of waking per day Av. for wk.	Range
	Av. for wk.	Range				
1st week	6.2	(4-7.5)	2.0	3.0	7.5	(6-9)
5th week	8.4	(7.5-10)	2.5	4.5	8.4	(6-11)
9th week	5.8	(4-8.5)	2.2	4.8	6.5	(5-8)
13th week	6.2	(4.5-7.5)	2.4	3.8	5.0	(4-6)
17th week	7.0	(5.5-8)	2.5	4.0	4.5	(4-5)
21st week	7.7	(7-9)	2.5	3.0	4.9	(4-6)
25th week	8.4	(7-10.5)	2.8	3.0	4.2	(3-6)
29th week	7.5	(6.5-8)	2.9	3.0	3.5	(3-4)
33rd week	9.5	(8-11.5)	4.0	5.0	4.0	(3-6)
35th week	9.7	(8.5-11)	4.1	6.5	4.2	(3-5)

duration of the longest wakeful periods for each week studied. As this baby matured he seemed to develop an increasing ability to sustain wakefulness just as he developed the ability to sustain sleep.

On the other hand, the single longest wakeful period during the week shows no consistent change from the first to the 33rd week. At 9 weeks it was 4.8 hours and at 33 weeks 5 hours, and at 1, 21, 25 and 29 weeks it was 3 hours (Table 2, Column 3). This probably indicates, as one would expect, that even in the earliest weeks the baby can have isolated prolonged wakeful periods, probably from unusual stimulation.

The frequency of the wakeful periods per day rather rapidly diminished each week (Table 2, Column 4). Correspondingly, of course, the frequency of separate sleep periods diminished. The increase in the average longest wakeful periods is not great enough to account for the increase

in total wakefulness, yet the frequency of wakeful periods decreased in number. Therefore, each individual wakeful period must be somewhat longer, as can be seen in the Behavior Day Charts.

The Behavior Day Charts 1 through 9, as presented, illustrate the day to day changes in the sleep and waking patterns of this infant. Each chart represents a four week period.

The first 16 weeks were characterized by a dramatic progression from what appeared to be chaotic sleep and wakefulness to a recognizable rather orderly pattern (Charts 1, 2, 3 and 4).

It is interesting to note that this baby did not always progress steadily towards greater organization. Often there was a period of increased disorganization just before a new arrangement of sleeping patterns. The first of these was between the end of the 3rd week and the end of the 6th week. During this time he was more wakeful with a greater total amount

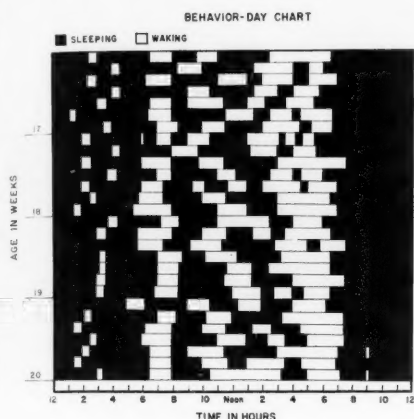


Fig. 5.

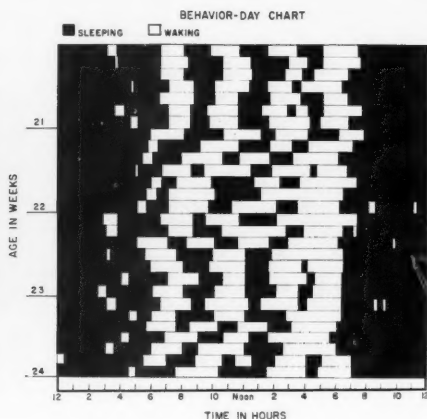


Fig. 6.

of time awake and also a greater frequency of wakeful periods than in the first three weeks of life. He was also more fussy (Tables 1 and 2 and Charts 1 and 2).

After the sixth week he was less restless and a diurnal sleep wakefulness pattern began to be apparent and became more obvious after the tenth week. From the 14th week on he slept consistently from 6 or 7 P.M. until midnight or beyond with rare relapses. At this time his afternoon schedule seemed to be quite regular also. His most disorganized period remained in the morning hours.

Crying was recorded on the Behavior Day Charts during the first 16 weeks and not after that. In the first 10 weeks crying was noted whenever there was a prolonged wakeful period and especially in the afternoon and evening. After 10 weeks the crying was less frequent and after 12 weeks there were long wakeful periods without crying, especially in the late morning.

This baby was breast fed for the first 10 weeks with some cow's milk supplements.

After 10 weeks he received only cow's milk feedings. Pureed foods and cereals were not started until after 16 weeks of age. In the first 2 weeks of life the frequency of feedings ranged from 6 to 8 per day with 7 the predominant number. After the sixth week frequency of feedings was 5 to 6 per day with 5 per day most common after the 11th week.

From the 16th week on the major changes were in the "night waking" between midnight and 4 A.M. as it is defined by Moore & Ucko (17), and in the day time sleep or naps occurring between 5 A.M. and 7 P.M.

The "night waking" will be discussed first. This boy had the least amount of "night waking" from the 27th through the 33rd week. In this period he awakened between midnight and 5 A.M. on 13 days of 42 or on an average of about two nights per week. During the 29th week (Chart 8) and for the first two days of the 30th week he did not awaken at all from 6 P.M. until 5 A.M. This was his most "settled" period and was the longest period of consecutive nights of prolonged



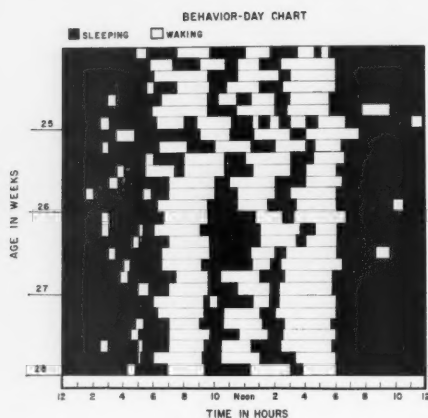


Fig. 7.

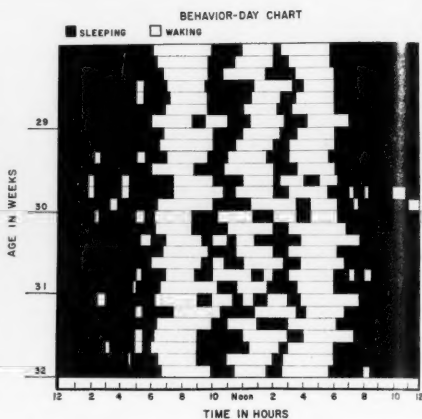


Fig. 8.

sleep. His daytime sleeping periods were also very regular during the 29th week. From the 33rd through the 35th week (Chart 9) his "night waking" was more frequent again and there were irregularities in his day time sleep as well.

Moore & Ucko (17) considered a baby "settled" if he slept through from midnight until 5 A.M. Occasional lapses were accepted as long as they were less than once a week. In their analysis of 104 cases they found that approximately 70% had settled by three months of age and 80% by 4 months. Nearly 10% were not settled by the end of the first year. A large proportion of relapses were noted between the 6th and 10th months. Hellbrügge *et al.* (10) studied infant sleep by checking the cribs in an infant nursery every other hour. For a group of 13 infants 5-8 months of age, checked for a period of 80 days, they found only 5% awake at 9 P.M. after a scheduled feeding. However, at 11 P.M., 1 A.M. and 3 A.M. 15% to 20% of the babies were awake and by 5 A.M. 35% had awakened.

The midday sleep or "naps" of this boy were irregular between 16 and 20 weeks (Chart 5). In general there were two or

three "naps" of irregular length during the day between 5 A.M. and 7 P.M. By the end of the 20th week and during the 21st week (Chart 6) the baby developed a very regular three nap schedule. This became irregular again from the 22nd through the 25th week when a new schedule of two regular daily naps began to appear. This schedule of two naps daily persisted from the 25th to the 33rd week (Charts 7, 8 and 9). In general the morning nap was longer than the afternoon nap.

Between the 33rd and 34th week (Chart 9) there was a shift to a long afternoon "nap". Gradually the morning nap was eliminated and the baby remained awake all morning and took a fairly long afternoon nap. The beginning of this is indicated in Chart 9 between the 34th and 35th week.

In general the more isolated restless days and nights noted in this boy's Behavior Day Charts could be correlated with "teething", immunizations, illnesses such as colds or diarrhea, or unusual family events such as extensive excursions

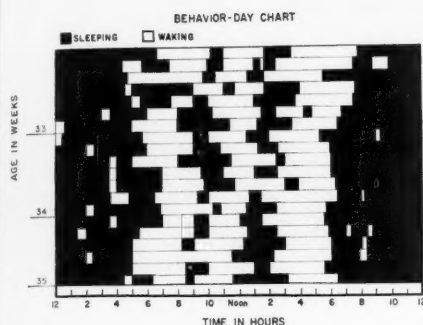


Fig. 9.

from the home or numerous visitors in the home.

Examples of this can be noted in the following weeks: 9th, a cold for 3 days; 10th, diarrhea on the 4th day; 12th, diarrhea on the 7th day; 26th, immunization on the 1st day; 27th, head cold for the entire week; 30th, immunization on the 6th day; and 35th, "teething".

The more lasting shifts in sleeping patterns could not be explained by observed events as noted above. In a general way they coincided with gross changes in the total development. For example, between 5 and 6 weeks a consistent smile could be elicited. At this time some evidence of a diurnal cycle became apparent and the extreme restlessness of the preceding 3 weeks subsided. A bigger change came after 12 weeks when the baby could lie awake and amuse himself by cooing and striking at his cradle gym with his hands. He began to play instead of crying when he awakened. At this time his sleep from 6 P.M. to midnight became consistent and undisturbed. After 20 weeks of age there was a major change in his posture during the daytime from the supine position to supported sitting,

and after 26 weeks he was sitting for prolonged periods with support. He played happily in this position, reaching for objects and transferring them from hand to hand. By 32 weeks he could sit for prolonged periods unsupported. During this period his total sleep pattern, day and night, was better organized than at any other time during this study.

After 32 weeks he began to become more mobile, changing from the seated to the prone position, and at 33 weeks he was crawling restlessly and getting himself into awkward positions. This mobility extended to a shift to the upright posture when at 34 weeks he began to pull himself to a standing position. This he enjoyed doing but he could not let himself down again and this made him fussy. Coincidentally, his day and night time sleep became somewhat irregular again. It also should be noted that the mother states he was teething during the 35th week.

Before a new organization of his sleeping patterns there often seemed to be a dissolution of the old one with a disorganized transition period. For example, the 5th, 22nd, 25th and 34th weeks. It would appear that this boy went through recurrent periods of rather complete disorganization as he passed from one major developmental stage to another. Gesell & Ilg (9) indicate that this is not uncommon throughout childhood.

Moore & Ucko (17) reported that in the first 3 months illness caused only brief disturbances in sleep, but later in the year an illness could cause a prolonged period of sleep disturbances. They also noted in the first 3 months external stimuli such as sharing a room or other sources of noise did not seem to influence settling. After the fourth month external circumstances seemed

to play a more important part in disturbing the baby's sleep. They did not discuss disruptions from changes in total developmental patterns.

Of additional interest is the observation that this baby showed evidence of a 23 hour rather than a 24 hour cycle until about the 16th or 17th week of life. Comparable feedings or wakeful periods on successive days seem to occur about one hour earlier, making a complete shift to the left on the charts in approximately 23 days. This is analogous to the case reported by Kleitman & Engelmann (15) with a 25 hour cycle and a similar daily shift in the opposite direction.

### Comments

There is a striking similarity in the general evolution of the patterns of sleep and wakefulness in this case and the other single cases reported as well as in the larger group studies previously mentioned. Moore & Ucko (17) noted this unity within their own study and commented as follows: "More surprising than the number of deviants from the norm, when all is considered, is the number who adhere to it in the face of difficulties."

Essentially the changes in the sleeping and wakefulness patterns from birth through at least the first eight months are not so much a decrease in the total amount of sleep but in the distribution of the periods of sleep and wakefulness. The neonatal infant has a significant amount of wakeful time in 24 hours, approximately 6-7 hours, as is indicated in this case and in a study of the sleep of 75 newborn infants during the first few days of life (19). However, this is broken up into many short periods and in this way appears to be negligible. Infants in the early weeks of life seem

to be incapable of sustaining a long period of sleep or a very long period of wakefulness. As they mature they acquire the ability to do both. Thus, the short periods of sleep consolidate into larger ones, especially in the night, and the short periods of wakefulness consolidate into larger ones, especially in the daytime. This is well documented in the study of Kleitman & Engelmann (15).

The total amount of sleep per day at each age level noted in this report is somewhat greater than the averages found by Kleitman & Engelmann (15). This could be accounted for by the fact that in this instance only the mother judged when the baby was awake or asleep, whereas in Kleitman & Engelmann's study this was checked more carefully with actograms. Thus, the periods when the baby was awake and active but not active enough or fussy enough to attract a mother's attention would be noted by Kleitman's technique and the total sleeping time recorded would tend to be shorter. Nevertheless, this child shows the same general patterns of development as indicated by Kleitman & Engelmann (15).

The exact nature of the process of development of sleep patterns as described is unknown. Moore & Ucko (17) concluded that babies do not "learn" to sleep all night in the sense that this is something that can be actively taught them by their parents. "What we have called the settling process, a form of learning at the level of biological adaptation, requires no consciously directed training by parents. There are indications that it is connected with fundamental physiological changes affecting the whole pattern of mental and physical activity."

Aserinsky & Kleitman (3) have studied eye movements and bodily movements of infants during sleep. They established a

basic rhythmic cycling of motility of approximately 60 min between peaks of motility. They suggest that the duration of sleep is likely to be one or a multiple of these motility cycles. In other words, an infant is most likely to wake up during the period of the motility cycle when he is most active. If he does not wake up at this time, he is likely to sleep through another motility cycle. The origin of this basic cycling of motility is not known. They considered it to be of internal origin, relatively independent of environmental factors.

Jouvet (12) has found in cats rhythmic bursts of electrical activity in the reticular formation of the pons that is related to deep sleep. This activity can be detected in intact animals with chronically implanted electrodes when they are in deep sleep, but is obscured in light sleep. The chronic mesencephalic preparation cats, on the other hand, have only this type of sleep, as indicated by the recordings from electrodes in the reticular formation of the pons, electromyographic recordings of the neck muscles and respiratory changes. The rhythmic discharging that causes this type of primitive sleep in cats seems to be independent of the external milieu.

One might postulate that the newborn infant has rhythmically induced sleep of short duration that is independent of his external environment. Superimposed on this is some ability on the part of the infant to respond to external stimuli and to be aroused, especially between discharges, and then to maintain this arousal despite a sleep cycle, or, as Aserinsky & Kleitman (3) state, maintain sleep despite a motility cycle. In any event, sustained arousal may be a function of higher central nervous system feedback to the reticular activating system (6), and sustained sleep may be partly dependent on the active suppression of the effectiveness of external

sensory experiences or habituation (11, 13, 20). As the young infant's central nervous system develops, it is increasingly capable of sustaining arousal and of screening out some sensory experience in order to maintain prolonged sleep. Further studies will be necessary to elucidate this problem. Such complex behavior as sleep and wakefulness probably cannot be explained sufficiently by such a simple postulate.

### Summary

A review and analysis of the sleep and wakefulness patterns of one boy from birth to 35 weeks of age has been presented. This boy's total sleeping time decreased gradually from 17.8 hours in the first week to 14.3 hours at 35 weeks. The most remarkable changes were in the increase in the duration of the sleep and waking periods. The average longest sleep period for the first week was 4.4 hours and for the 29th week 11.7 hours. The frequency of wakeful periods dropped from an average of 7.5 in the first week to 4.2 in the 35th week. A diurnal sleep pattern is apparent in the Behavior Day Charts from the sixth week on and very definite after the 12th week. The sleep pattern noted in this case are essentially the same as reported in the literature cited. Essentially, all have noted predominantly a lengthening of single sleep and wakeful periods with maturation in the first year, rather than a marked reduction in the total amount of sleeping time.

Possible neurophysiological explanations for the changes in sleep patterns are discussed.

## References

1. ABRAMSON, H. A. (Ed.): Problems of Consciousness; Transactions of the First Conference 1950, Josiah Macy, Jr., Foundation, 1951.
2. ADRIAN, E. D. and JASPER, H. H. (Eds.): Brain Mechanism and Consciousness, Charles C. Thomas, Springfield, Illinois, 1954.
3. ASERINSKY, E. and KLEITMAN, N.: A motility cycle in sleeping infants as manifested by ocular and gross bodily activity. *J Appl Physiol*, 8: 11, 1955.
4. BÜHLER, C. and HETZER, H.: Soziologische und psychologische Studien über das erste Lebensjahr. Jena, Gustav Fischer, 1927.
5. DEBRÉ, R. and DOUMIC, A.: Le Sommeil de l'Enfant. Presses Universitaires de France, Paris, 1959.
6. FRENCH, J. D., HERNANDEZ-PEON, R. and LIVINGSTON, R. B.: Projections from cortex to cephalic brain stem (reticular formation) in monkey. *J Neurophysiol*, 18: 74, 1955.
7. GESELL, A. and AMATRUDA, C. S.: Embryology of Behavior, Harper, New York, 1945.
8. GESELL, A. and ILG, F.: Feeding Behavior of Infants, Lippincott, Philadelphia, 1937.
9. — Infant and Child in the Culture of Today, Harper, New York, 1943.
10. HELBRÜGGE, T., LANGE, J., and RUTENFRANZ, J.: Schlafen und Wachen in der kindlichen Entwicklung, Beihefte zum Arch. für Kinderheilkunde, No. 39, Ferdinand Enke, Stuttgart, 1959.
11. JASPER, H. H.: (Chapt. 15). Recent advances in our understanding of the ascending activity of the reticular system. In the Henry Ford Hospital Symposium, Reticular Formation and the Brain, Detroit, 1957.
12. JOUVET, M., MICHEL, F. and COURJON J.: Sur un stade d'activité électrique cérébrale rapide au cours du sommeil physiologique. *C R Soc Biol*, 153: 1024, 1959.
13. JOUVET, M. and MICHEL, F.: Aspects électro-encéphalographiques de l'habituatation de la réaction d'éveil. *J Physiol (Par)*, 51: 489, 1959.
14. KLEITMAN, N.: Sleep and Wakefulness, as Alternating Phases in the Cycle of Existence. Univ. of Chicago Press, Chicago, 1939.
15. KLEITMAN, N. and ENGELMANN, T. G.: Sleep characteristics of infants. *J Appl Physiol*, 6: 269, 1953.
16. MAGOUN, H. W.: The Waking Brain, Springfield, Charles C. Thomas, Illinois, 1954.
17. MOORE, T. and UCKO, L. D.: Night waking in early infancy. *Arch Dis Child*, 32: 333, 1957.
18. MULLIN, F. J.: Development of the diurnal temperature and motility pattern in a baby. *Amer J Physiol*, 126: 589, 1939.
19. PARMELEE, A. H., JR., SCHULZ, H. R. and DISBROW, M. A.: Sleep patterns of the newborn; To be published in *J Pediat*, 57: 1961.
20. SHARPLESS, S. and JASPER, H. H.: Habituation of the arousal reaction. *Brain*, 79: 655, 1956.
21. SIMSARIAN, F. P. and MCLENDON, P. A.: Further records of the self-demand schedule in infant feeding. *J Pediat*, 27: 109, 1945.

Received June 20, 1960

Accepted July 21, 1960

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## CASE REPORT

### Icterus and Xanthomatosis

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Xanthomatous changes in the skin are rare in children and comparatively little has been published on the subject from pediatric quarters. Crocker (5) and Moriquand (14) independently deal with xanthomatosis in children in their reviews, and it is apparent that the etiologic and pathogenetic possibilities are many. Disturbances of the liver and bile ducts may cause xanthomatosis in children. No conclusive proof that acute virus hepatitis has been the causative factor in xanthomatosis has yet been reported in medical literature. In fact, Thannhauser (17) categorically maintains that the two do not occur together. On the other hand, Magnusson, Josephsen & Tungland (13) in 1956 reported a case of xanthomatosis associated with icterus in a 7 year old boy. They considered an acute viral hepatitis to be the most likely etiology. We have had occasion to study an almost identical case in the Pediatric Department, Oslo University Hospital, and thought it worthy of notice.

#### Case report

The patient is a boy, born April 25, 1956, who has previously been in good health. He comes from a healthy family none of

whom have cutaneous xanthomas. The parents and two other siblings are living and in good health. The pregnancy, delivery and neonatal period were uneventful. No nutritional problems. Psychomotor development normal.

The first manifestation of the present disease occurred at the age of two years. The boy became listless, irritable, somewhat feverish and would not eat. After about a week, dark urine, clay-coloured stools and jaundiced skin were observed. Information was obtained that no other case of jaundice had occurred in the district at that time. As for the possibility of inoculation hepatitis, it was reported that the patient had had his last injection of polio vaccine in November 1957.

During the following 4 to 5 weeks, the icterus gradually became pronounced, and the patient developed pruritus. He stayed at home, partly confined to bed, and was given a light diet but no other treatment. In the beginning of May, he was admitted to the Department for Epidemic Diseases, Central Hospital, Trondheim. Marked jaundice, positive Gmelin, and negative Schlesinger reaction in the urine were demonstrated. The patient was discharged from the hospital after 8 days with the diagnosis of acute hepatitis. His condition remained the same. The jaundice persisted without change for about 8 weeks after discharge from the hospital, and the boy continued to be listless and uncomfortable from the itching. From the beginning of July, the patient improved,



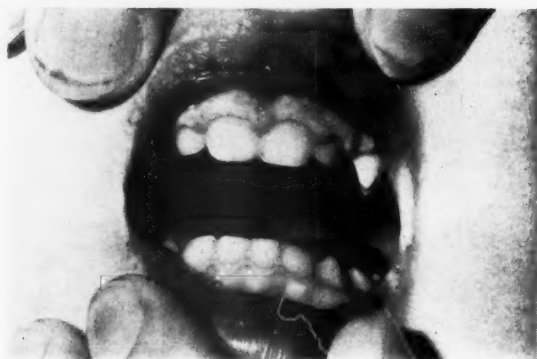


Fig. 1.

the icterus decreased and the urine became lighter. At the same time, however, an eruption of yellowish-white nodules on hands and feet was observed. When the patient was examined by the referring pediatrician at the end of July, a moderate icterus was still present, and there was a general eruption of xanthomas in addition. The patient was therefore referred to the Pediatric Department, Oslo University Hospital.

On admission, Aug. 13, 1958, the boy was two and a half years old. He was thin, somewhat pale, afebrile and not icteric. The skin was spotted with small yellowish-white nodules, particularly along the lines of palms and soles. Efflorescences of the oral mucosa, specially on the gingivae, were also dem-

onstrated (Fig. 1 and 2). The liver was considerably enlarged, extending to the umbilical level, and was slightly tender. The surface was smooth and firm. Splenomegaly was not demonstrable. No other pathologic findings were brought to light by the clinical examination.

Laboratory findings: Routine chemical and microscopic examination of the urine revealed nothing abnormal. The serum was markedly turbid. Hemoglobin: 75 %. Sedimentation rate: 84 mm per hour. Leucocytes: 5100  $\text{mm}^3$ , distribution normal. Erythrocytes: 3.84 millions per  $\text{mm}^3$ . Bilirubin (total): 0.6 mg%. Alkaline phosphatase: 35.7 Bodansky units. Takata's reaction negative. Serum proteins: Albumin 3.7 g%.



Fig. 2.



Globulin 4.6 g%. Alfa 2 globulin 1.3 g%. Beta globulin 1.8 g%. Gamma globulin 1.2 g%. Total lipids: 5340 mg% (normally 400-700). Total cholesterol: 1742 mg% (Table 1).

Roentgenographic examination of the skeleton revealed considerable osteoporosis. A cholangiogram disclosed normal gall bladder and good excretion of contrast. Biopsy of the skin: Xanthomatosis.

The patient was placed on a diet low in cholesterol and given meticorten with the dosage of 5 mg four times a day. In the course of 12 weeks, this was reduced to 2.5 mg twice a day. Subsequently, the xanthomatosis became less marked, the liver less enlarged and the biochemical changes showed steady improvement (Table 1). He was discharged from the hospital on November 3, 1958 and instructed to follow a diet low in cholesterol and continue to take meticorten 2.5 mg twice daily.

The patient was later admitted in February, 1959 and September, 1959 for re-examination. For practical reasons, it had not been possible to continue his low cholesterol diet, and the meticorten medication was discontinued in March, 1959. He had enjoyed excellent health all the time. On the final examination, the xanthomas

were considerably reduced, had completely disappeared from the face and mucous membranes, but were still present in both palms and on buttocks. The liver was no longer palpable. Laboratory findings of significance are shown in Table 1.

### Discussion

A simultaneous occurrence of icterus and xanthomatosis raises etiologic, pathogenetic and diagnostic considerations which have long been a controversial subject. Depending on the author's view as to the etiology and pathogenesis opposite conclusions have been drawn at different times, particularly as to whether the liver and bile duct disturbance or the xanthomatosis is the primary cause of the condition.

On the basis of recent studies and the findings in this particular case, the relation between icterus and xanthomatosis in our patient will be discussed below.

The diagnosis of acute viral hepatitis can usually be made with reasonable

TABLE 1. *Laboratory data during the course of the disease.*

	1958 Aug. 15	1958 Aug. 21	1958 Sept. 9	1958 Oct. 23	1959 Febr. 26	1959 Sept. 16
Total lipids	5310 mg %	4345 mg %	1880 mg %	1125 mg %	860 mg %	710 mg %
Neutral fat		1317 mg %	708 mg %		360 mg %	290 mg %
Free fatty acids		100 mg %	200 mg %	130 mg %		
Total cholesterol	1712 mg %	1248 mg %	624 mg %	250 mg %	246 mg %	176 mg %
Phospholipids		1680 mg %	348 mg %	320 mg %	254 mg %	235 mg %
Alkaline phosphatase (Bodansky)	35.7		12.7		5.1	9.3
Thrombol	0.15		0.13	0.16	0.05	0.08
Taibata	Negative		Negative			Negative
Prothrombin-proconvertin time in % of normal	120 %			125 %	83 %	115 %
Bilirubin	0.6 mg %				0.8 mg %	
Total serum proteins	8.3 g %		7.3 g %	7.2 g %	6.9 g %	7.1 g %
Sedimentation rate	84 mm per hour		12 mm per hour	23 mm per hour		11 mm per hour

certainty from the clinical data and simple laboratory examinations. It is well known, however, that the disease can appear in different forms. One of these is the so-called cholangitic form, characterized by protracted icterus and pruritus, and laboratory findings that point to an obstructive disturbance rather than to parenchymatous injury. The patients recover slowly, with complete recovery usually occurring within a year.

Our patient presented just this picture. Icterus and pruritus persisted for 4 months. The alkaline phosphatase was increased, electrophoresis of the serum proteins revealed findings that indicated an obstructive disturbance, while routine tests for a parenchymatous injury revealed nothing abnormal. Apart from the occurrence of xanthomatosis, the convalescence was protracted and the liver did not return to normal size for about a year. There were no reports of other cases of jaundice in the patient's home district, but the possibility of hepatitis resulting from inoculation is present, as the patient was vaccinated against poliomyelitis 4 months before the onset of the disease.

This type of hepatitis does not seem to be rare. Eliakim & Rachmilewitz (7) present a study of 104 cases of acute hepatitis. Nearly one fifth of the patients showed the picture of the cholangitic type of illness. In these, the condition was practically indistinguishable from that of obstructive jaundice. Liver biopsy is a most helpful diagnostic measure (4), but unfortunately we do not have this conclusive proof in our patient. The course of the illness, however, makes the diagnosis viral hepatitis of cholangitic type highly probable.

The main point of interest is the second stage of the disease, the development of the xanthomatosis, and the relationship between this and a hepatitis of cholangitic type. As mentioned in the introduction, it is well known that many liver and bile duct disturbances can engender secondary xanthomatosis. This phenomenon has been reported in liver glycogenosis (19), toxic hepatitis (6), congenital dysplasia of the interlobar bile ducts (2, 5, 8, 11), protracted bile stasis caused by extrahepatic occlusion of varied etiology (3), and in primary biliary cirrhosis (11, 15, 16). These conditions have the following common traits: a more or less chronic bile stasis serum lipids which are demonstrably elevated, and liver function tests which remain normal for a long time. Ahrens & Kunkel (1) found that patients with total lipid serum values of more than 2000 mg %, lasting for more than 6 months, always developed xanthomatosis, whereas xanthomatosis did not develop in those with serum lipid values below 1300 mg % no matter what the duration.

Our patient presented all of these characteristics. The clinical evaluation and, even more, the course of the patient's illness do not, however, point to the presence of any of the above mentioned liver and bile duct disturbances. During the first part of the first hospitalization, the picture presented came closest to that of xanthomatous biliary cirrhosis. This somewhat nebulous syndrome, today interpreted as a primary liver disease of various etiology (3, 6, 11, 16), is a chronic condition with remittent attacks of jaundice and a poor long term prognosis. This disease can thus be ruled out as a diagnostic possibility in our case.

Primary biliary cirrhosis can, as mentioned above, give rise to secondary xanthomatosis. In this connection, it should be noted that primary biliary cirrhosis may develop as a consequence of acute cholangitic hepatitis (18). It seems likely that the same pathological mechanisms can be at work in both stages. Therefore it is logical to assume that xanthomatosis can evolve from an acute as well as a chronic phase, provided the prerequisites for xanthoma formation are present.

It is interesting to note that in all previously reported cases with secondary xanthomatosis caused by liver and bile duct disturbances, the plasma has always been clear. The rise in the serum lipids was entirely due to an increase of cholesterol and phospholipids while neutral fat concentration was normal. In our patient, the plasma was turbid, owing to a marked increase in the neutral fat concentration (Table 1). This finding may suggest the possibility of a particular primary disturbance in the lipid metabolism, i.e. idiopathic familial hyperlipemia. The plasma in this condition is highly lipemic, all lipid fractions are considerably elevated and xanthomatous deposits in the main biliary ducts may lead to bile stasis with jaundice. The clinical picture is then indistinguishable from secondary xanthomatosis caused by primary liver and bile duct disturbances (16). These are precisely the differential

diagnostic possibilities emphasized by Magnusson and his coworkers (9, 13). In the present case, the possibility of primary xanthomatosis seems completely unlikely. There is nothing to support a hereditary predisposition in the family history, the xanthomatous skin changes did not occur until the icterus had practically subsided, and roentgen contrast examination of the bile ducts revealed good excretion. It seems unthinkable that resorption of primary xanthomatous deposits in the bile ducts should take place in the presence of severe cutaneous xanthomatosis and while the serum lipids were markedly elevated. The most likely conclusion seems to be that the patient reported here has had a primary liver disease, probably a hepatitis of cholangitic type, with development of secondary xanthomatosis.

### Summary

The clinical course of a two and a half year old boy with icterus, hepatomegaly, xanthomatosis and marked elevation of the serum lipids is described. In the process of this disease, these clinical and biochemical signs slowly disappeared and the patient seems to have regained complete health. The diagnostic problems are discussed. It is concluded that the patient suffered from a primary liver disease, most likely viral hepatitis of the cholangitic type, with development of secondary xanthomatosis.

### References

1. AHRENS, E. H. Jr. and KUNKEL, H. G.: Relationship between serum lipids and skin xanthomas in 18 patients with primary biliary cirrhosis. *J Clin Invest*, 28: 1565, 1949.
2. AHRENS, E. H., HARRIS, R. C. and MAC
- MAHON, H. E.: Atresia of the intrahepatic bile ducts. *Pediatrics*, 8: 628, 1951.
3. AHRENS, E. H. Jr., PAYNE, H. G., KUNKEL, W. J., EISENMENGER, W. J. and BLONDHEIM, S. H.: Primary biliary cirrhosis. *Medicine*, 29: 299, 1950.

4. CALL, E. A. and BRAUNSTEIN, H.: Hepatitis with manifestations simulating bile duct obstruction. *Amer J Clin Path*, 25: 1153, 1955.
5. CROCKER, A. C.: Skin xanthomas in childhood. *Pediatrics*, 8: 573, 1951.
6. DUBIN, I. N.: Cholestatic hepatitis. *Bull NY Acad Med*, 32: 396, 1956.
7. ELIAKIM, M. and RACHMILEWITZ, M.: Cholangitic manifestations in virus hepatitis. *Gastroenterology*, 31: 369, 1956.
8. HAAS, L. and DOBBS, R. H.: Congenital absence of the intrahepatic bile ducts. *Arch Dis Child*, 33: 396, 1958.
9. JOSEPHSEN, J. O.: Xanthomatose og levergalleveislidelser. *Nord Med*, 55: 77, 1956.
10. MACMAHON, H. E.: Die biliäre Xanthomatose bei Erwachsenen und Kindern. *Virchow Arch Path Anat*, 321: 664, 1952.
11. MACMAHON, H. E. and THANNHAUSER, S. J.: Xanthomatous biliary cirrhosis (a clinical syndrome). *Ann Int Med*, 30: 121, 1949.
12. — Congenital dysplasia of the interlobular bile ducts with extensive skin xanthomata: Congenital acholangic biliary cirrhosis. *Gastroenterology*, 21: 488, 1952.
13. MAGNUSSON, R., JOSEPHSEN, J. O. and TUNGLAND, B.: Icterus og xanthomatose. *Nord Med*, 55: 83, 1956.
14. MORIQUAND, CL.: Les xanthomes cutanés chez l'enfant et le nourrisson. *Pédiatrie*, 2: 163, 1957.
15. NEZELOF, R. J. Ch., RIBIERRE, M. and JOE, J.-C.: Ictère cholestatique chronique par cholangiolite et péricholangiolite (syndrome de MacMahon) chez un enfant de 18 mois. *Ann Pediat (Par)*, 52-53: 2123, 1959.
16. SHAY, H. and HARRIS, C.: Changing concepts of "xanthomatous biliary cirrhosis". *Amer J Med Sc*, 223: 286, 1952.
17. THANNHAUSER, S. J.: Lehrbuch des Stoffwechsels und der Stoffwechselkrankheiten. Georg Thieme Verlag, Stuttgart, 1957.
18. WATSON, J. C. and HOFFBAUER, F. W.: The problem of prolonged hepatitis with particular reference to the cholangitic type and the development of cholangitic cirrhosis of the liver. *Ann Int Med*, 25: 195, 1946.
19. ZAKOU, S. J., OYAMADA, A. and ROSENTHAL, I. H.: Eruptive xanthoma and hyperlipemia in glycogen storage disease. *Arch Dermat Syph*, 67: 146, 1953.

Received June 10, 1960

Accepted July 15, 1960

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## CASE REPORT

### Hereditary Renal Dysplasia and Blindness

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Hereditary progressive renal disease was first described by several authors around 1900. Mitchell (12) reviewed the literature in 1930. Since then a similar clinical picture has been reported combined often with abnormalities in other organs.

Ryersbach (13) described a neurogenic deafness. Hawkins & Smith (7) reported various skeletal and skin abnormalities in 36 cases. These belonged to 4 generations of the same family. Congenital cataract was present in 3 of their cases. Sohar (15) described 10 cases among whom 2 had *spherophakia* and 2 had congenital cataract. Goldbloom (6) reported 3 siblings aged 5, 10 and 18 years, all of whom died with uremia. Two had reduced hearing, and one of these also an anterior subcapsular cataract. In no case has histological examination of the eyes been performed.

In the few autopsied cases the renal lesion was found to be pyelonephritis. The pathogenesis, however, was unclear until Marshall (11) and Ericsson & Ivemark (3, 4). Ivemark, Oldfeldt & Zetterström (8) observed that pyelonephritis may develop on the basis of renal dysplasia.

The present report deals with 2 blind siblings who died with renal failure at 8 and 10 years of age. In one case a postmor-

tem examination including the eyes was performed. The parents are unrelated. Several members of the fathers family have died of "stroke" at the age of 60-70 years. Three of the mother's siblings have died, one from diabetes, one from multiple sclerosis and one from lymphosarcoma. No other hereditary disease or abnormalities are known in the family.

#### Case Reports

*Case 1.* T.A., male, born May 29, 1939. The pregnancy was uncomplicated and delivery was uneventful. Birth weight 4350 g. When he was 3 months old the parents noticed that his eyes did not follow light or objects, but he reacted to strong light stimulation by closing his eyes. The psychomotoric development was markedly retarded. At age 2 years he was admitted to the Eye Department and Pediatric Clinic of Rikshospitalet, Oslo.

His musculature was hypotonic, and he showed both mental and motor retardation. The eyes were slightly sunken in, there was nystagmus, and he did not focus on any object. The pupils reacted sluggishly to light. Ophtalmoscopy revealed normal eye-grounds. The following years an increasing mental retardation occurred. He could only utter a few words; he was confused and

agitated, had a poor appetite and vomited frequently. He was cared for in a home for mentally retarded children.

At 7 and 7½ years old he was again seen in the Pediatric Clinic. Weight 14.6 kg, height 107 cm, blood pressure 100/60. The skin was pale and dry with petechiae. Examination of the eyegrounds was normal as was his hearing. This condition grew increasingly worse. Clonic seizures occurring particularly at night began and at 8 years of age he died in a local hospital following a short period of vomiting, diarrhea and repeated convulsions.

**Laboratory findings:** Urinalyses were normal when he was 2 years old. At 7 and 7½ years proteinuria was repeatedly present (Esbach was ¼%) and specific gravity was invariably below 1.010. Benzidine reaction was negative, and microscopy showed a few white blood cells and granular casts.

**Blood:** At 2 years of age the Hb. was 82% and sedimentation rate 2 mm. At 7 years Hb. was 50%, erythrocytes 2.36 mill/mm<sup>3</sup>, leucocytes 9,300 with normal differential count. Bone marrow biopsy showed scarce erythropoiesis. Serum iron was 92 gamma%. Bleeding time, coagulation time and prothrombin time and clot retraction were normal. Pneumoencephalography was unsuccessful. ECG and X-ray of the heart were normal.

**Case 2.** T.A. female born November 7, 1950 following an uncomplicated pregnancy and delivery. Birth weight was 3830 g. No neonatal complications occurred. Her growth and motor development were normal. She was said to have fixed on objects at 2-3 months of age. However, at 5½ months of age she was admitted to the Pediatric Clinic, Rikshospitalet, because of reduced vision. She did not follow any object with her eyes. The eyegrounds and the remaining examination were normal.

In the following years she continued to develop normally, except for her visual defect. At 7 years complaints of weakness, abdominal pains and nausea began. She was found to be anemic and was readmitted to the Pediatric Clinic. Physical examination revealed a pale and thin girl who weighed

24 kg and measured 122 cm. Some light perception was still preserved. The pupils were dilated and reacted sluggishly to light. Ophthalmoscopy showed normal optic disc. The macular regions appeared normal, the vessels showed no pathological changes and in the periphery a diffuse pigmentation was seen. The refracting media were clear. Her hearing was normal. Because of the anemia she received blood transfusions, and cortisone was tried without effect.

Her condition remained unchanged for 2 years. At 9 years of age she became increasingly fatigued with headache and vomiting, her gait was spastic and she had periodic clonic jerks. She became dyspnoeic and stuporous and was readmitted to the Pediatric Clinic. Her skin was extremely pale with multiple petechiae and ecchymoses. Blood pressure was 170/100. Blood and saline transfusions were given without effect, and she died the next day following repeated convulsions.

**Laboratory investigations:** At 5 months of age her blood and bone marrow were normal.

Seven years old: **Urinalyses:** The protein reactions were positive and the specific gravity was below 1.010, even after a concentration test. Microscopy revealed 8-10 white blood cells per visual field as well as scattered granular casts. Urine cultures were sterile.

**Blood:** Hb. was 58%, erythrocytes 3.70 mill/mm<sup>3</sup>, reticulocytes 3%, thrombocytes 120,000-210,000/mm<sup>3</sup>, leucocytes 13,200/mm<sup>3</sup> with a shift to the left of the differential cell count. Sedimentation rate was 28 mm. The bone marrow was normal. Serum iron 69 gamma%, creatinine in serum was 3.5 mg%, urea 132 mg%, Co<sub>2</sub>-combining power 4.7 vol%, calcium 9.8 mg%, phosphorus 5.4 mg% and alkaline phosphatase 14 units (Busch). The dye test was negative and the antistreptolysin titer 700. Electrophoresis showed an increase of alpha<sub>2</sub>-globulins but was otherwise normal. X-ray of the skeleton and retrograde pyelography were negative. Electroencephalogram showed no definite pathological changes.

Nine years old: Hb. was 24%, sedimentation

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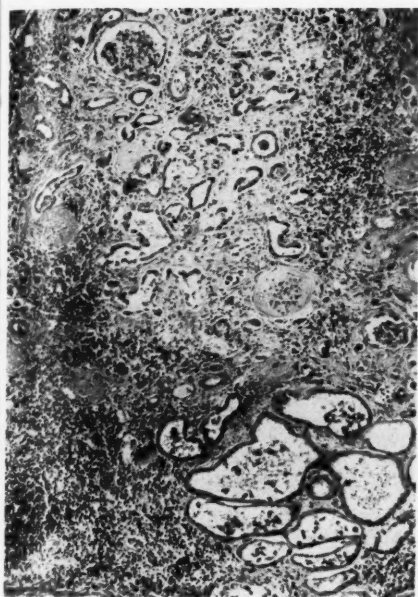


Fig. 1. Section from the kidney, showing loss of convoluted tubules. Groups of dilated tubules are seen. Only a few glomeruli are preserved. Lymphocytic infiltrations. Increased connective tissue. Hematoxylin-eosin.  $\times 80$ .

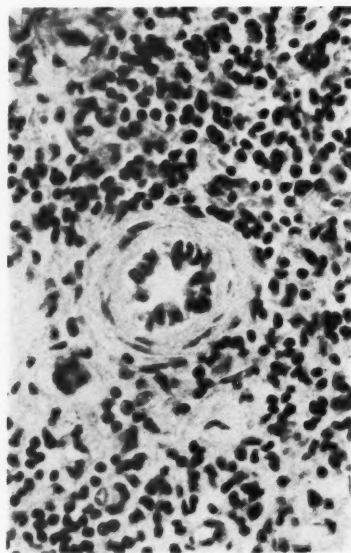


Fig. 2. Dysplastic renal ductule lined by cubocylindrical epithelium, and surrounded by concentric rings of connective tissue. Hematoxylin-eosin.  $\times 400$ .

tion rate 20 mm, urea 424 mg%,  $\text{CO}_2$ -combining power 10.7 vol% and chlorides 105 mEq.

**Autopsy.** The pertinent findings were: Small amounts of serous fluid in the pleural cavities (ca. 20 ml), in the pericardial cavity (5 ml) and in the peritoneal space (100 ml). The lungs were edematous and hemorrhagic. The heart was slightly enlarged, weighing 170 g, and there was some hypertrophy of the left ventricular wall.

The kidneys weighed 28 g and 27 g. They appeared shrunken, and their surface was slightly granular. The cut surface had a yellowish tinge, and the cortical substance varied in width from 1-3 mm. Pelves, ureters and bladder were normal.

Microscopic examination of the kidneys (Fig. 1) revealed distorted cortical structures, primarily due to atrophy of the tubules. Only a few scattered groups of dilated

tubules were present, filled with a homogeneous eosinophilic substance. The majority of the glomeruli showed either pericapsular fibrosis of varying degrees, or they were completely hyaline. A number of glomeruli were, however, still well preserved. A widespread and heavy infiltration of lymphocytes and plasma cells was seen in the cortex, far less in the medulla. The collecting tubules were reduced in number, and some fibrosis was found.

In the peripelvic tissue, perivascularly and scattered throughout both medulla and cortex areas of renal dysplasia were present, characterized by ductules lined with cuboidal epithelium and coated with a distinct rim of concentrically arranged connective tissue fibers sometimes surrounded by lymphocytes (Fig. 2). Groups of them were seen in fibrous areas without any chronic inflammation. Other structures indicating



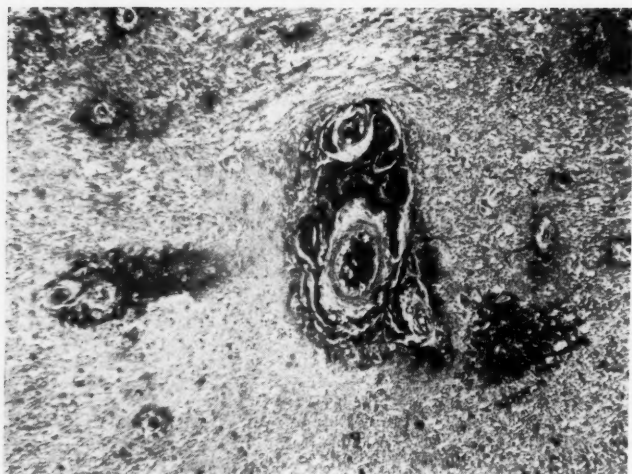


Fig. 3. Brain stem with edema and small hemorrhages. Fibrinoid material in the perivascular spaces and deposited in the surrounding brain tissue. P.T.A.H.  $\times 80$ .

primitive glomeruli were also found. The parietal layer was coated by columnar or cuboidal epithelial cells. The tufts were bizarre and made up by cuboidal, dark staining epithelial cells and deposition of mineral salts occurred.

Gross examination of the *brain* showed only some flattening of the gyri. The weight was 1400 g. After 14 days fixation in 10% formalin the brain was cut. A few pin-prick-sized hemorrhagic spots were present in pons and tegmentum. Microscopically these areas showed an edematous tissue. There was some swelling and proliferation of the endothelial cells as well as of the adventitial connective tissue. The vessels were surrounded by a mostly cell-free, slightly eosinophilic staining substance, bordered by a dense fibrinoid material deposited in the brain tissue (Fig. 3). By Mallory's connective tissue staining the material stained brightly red or more orange. It was PAS positive and stained dark purple-blue by P.T.A.H. A few perivascular hemorrhages and glioses were also found. The optic pathways, such as the superior collicle, the geniculate bodies, the optic radiation and area striata were normal.

The posterior parts of the *eyes* were removed through the orbital roof with the optic nerves. They were fixed in Stieves fluid and embedded in paraffin.

The two eyes showed nearly identical microscopical pictures. The sclerae and choroidal layers were normal. The pigment epithelium was preserved, but the pigment was rather scarce. There was some scattering of pigmented cells in the choroidal layers. The retina was separated from the pigment epithelium. There was a marked reduction of cells. In the macular regions one single layer of rather large cells of an epithelial appearance replaced the outer granular layer (Fig. 4), probably representing the ependymal cell layer from which the rod and cone cells normally develop. The external limiting membrane could be seen separating the cell bodies from their cone shaped extensions. No rod cells could be identified. In more peripheral parts of the retina the outer granular layer contained only a few irregularly distributed cells (Fig. 5), and a few pigment-laden cells were scattered among them, sometimes gathered around small vessels. The plexiform layers as well as the

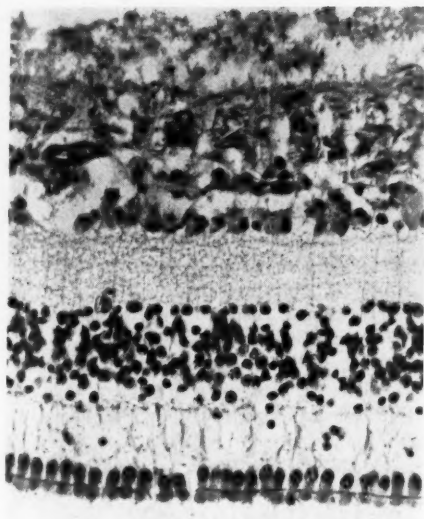


Fig. 4. Macular region of the left eye. The outer granular layer is represented by one single layer of cells. The external limiting membrane is preserved. The inner granular layer is fairly well developed, and a few ganglion cells are seen. P.T.A.H.  $\times 320$ .

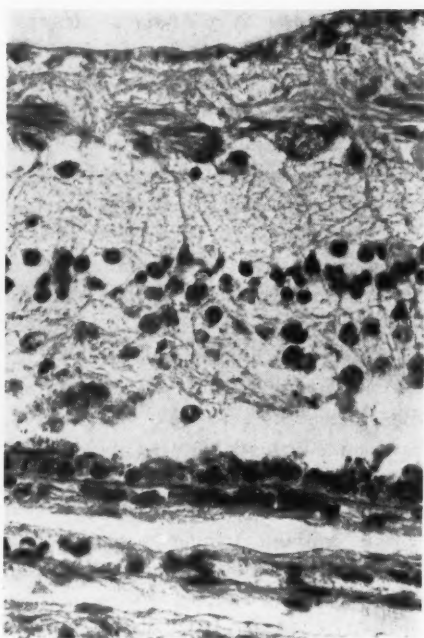


Fig. 5. The retina, pigment epithelium and chorioidal layers in peripheral part of the right eye. The various retinal layers are severely reduced, only the fiber layer is thickened. P.T.A.H.  $\times 460$ .

inner granular layer were markedly atrophic, but not to the same degree as the outer granular layer. Some ganglion cells were preserved. The nerve fiber layer was enlarged by growth of glial fibers, and contained well preserved vessels of the usual size and shape. The optic nerve was normal.

### Discussion

In the previously reported cases of hereditary nephropathy combined with deafness, or less commonly ocular disturbances, the true nature of the renal lesions seems obscure. In the few cases coming to autopsy the histological examination has revealed wide-spread pyelonephritis.

Goldbloom *et al.* (6) emphasized that the pathological findings probably represented end stages completely masking the underlying primary renal lesion. In an attempt to explain the peculiar susceptibility of these individuals to pyelonephritis these authors suggested multifocal hypoplasia or malformations as the possible primary cause. In the absence of anatomic abnormalities an inherited enzymatic defect was considered. Studies by Marshall (11) and more recently by Ericsson & Ivemark (3, 4) provide evidence for the assumption that patients with dysplastic kidneys are highly susceptible to pyelonephritis.

In our Case 2, wide-spread chronic pyelonephritis with almost complete distortion of the renal parenchyma was evident. Primitive ducts or cartilage held to be indisputable evidence of dysplasia were not seen. However, other structures, supporting the diagnosis of dysplasia as reported by Ericsson & Ivemark (3, 4) were common in the specimens examined. These structures were ductules lined by cuboidal epithelium and surrounded by distinct rings of concentric connective tissue bands. Formations apparently representing primitive glomeruli were also encountered. Ericsson & Ivemark (4) were able to show that such ductules originated in the medulla and ended blindly beneath the renal capsule without joining uriniferous tubules. The primitive or abnormal glomeruli were isolated or connected with atrophic dilated proximal tubules with no outlet. In their opinion, a kidney should be regarded as dysplastic when such ductules are found. The primary lesion in the present case is therefore believed to be renal dysplasia.

The degenerative changes of the vessel walls in the brain stem are believed due to the uremia. The vascular changes are of the same kind as those found in the kidneys. The perivascular transudate indicates a disturbed blood-brain barrier, causing edema and deposit of an albuminous material in the brain substance. This apparently resulted in a glial reaction around the vessels. The location of uremic changes in the brain stem is not unusual (2) and the wide-spread distribution in the pons and reticular substance of the brain stem accounts well for the convulsions, coma and death.

Congenital blindness has been the subject of recent large scale investigation. Alström & Olson (1) found that 10% of the blind children in Sweden from 1897 to 1948 belonged to the group classified hereditarily congenital. This group included various clinical diagnoses such as retinoblastoma, congenital cataract and atrophy of the optic nerve. The ophthalmoscopic picture may be normal in early cases, but more often a dusty pigmentation was found which increased with age. ERG was negative in most of the cases. Cataract and keratoconus were present, increasing in frequency with age. No increased frequency of mental deficiency compared to the rest of the population was found. Genetic studies showed a monohybrid recessive autosomal heredity.

Schappert-Kimmijser *et al.* (14) studied 227 cases of amaurosis congenita in the Netherlands, and their descriptions were much in accordance with those of Alström & Olson. ERG could not be separated from those of retinitis pigmentosa. They also found an autosomal recessive heredity.

Sorsby & Williams (16) described 9 patients with retinal aplasia in 2 families. Several also had mental retardation. These authors separated the condition from retinitis pigmentosa on the basis of the different clinical record, but suggested that the aplasia was related to retinitis pigmentosa, possibly caused by a more severe developmental deficiency. They referred to Keeler's investigations (9, 10) in the house mouse. Based on histological examinations of the eyes and electromotive force generated through stimulation by light Keeler found mice with hereditary blindness mainly due to a reduction of the rod cells. The reduction was present in varying degrees, and he found that the pigment epithelium sometimes broke through the external limiting membrane, and scattered pigmented cells were found between the cells in the outer layers of retina.

Histological examination in human cases of retinal aplasia has not been found in the literature. The histological picture in the

present case is very similar to the description by Keeler (9, 10) in mice, and it has also features common with retinitis pigmentosa. In our case the displaced pigment was rather scarce. Early cases of this disease have, however, been described as retinitis pigmentosa sine pigment (17). Our impression that the degeneration starts in the outer granular layer, and spreads inwards, is also in accordance with Wolffs descriptions of retinitis pigmentosa. As to Keeler's descriptions of the rodless mice the present case is very similar to his most advanced aplasias with depletion of rods and one layer of cones left.

The present case seems to display two different congenital defects. Both dysplastic kidneys and retinal dysplasias have been described as genetically determined abnormalities. The dysplastic kidney has been found combined with several other anomalies, most often with neurogenic deafness. The retinal dysplasias sometimes were combined with mental deficiency. Whether the cataract described in cases of renal dysplasia might have been combined with retinal changes is not known. It

should, however, be remembered that both cataract and keratoconus are frequently combined with retinal aplasia. Alström & Olson (1) concluded that the gene carrying the anomaly influenced both retina, lens and cornea.

To the authors knowledge the occurrence of hereditary renal disease and congenital blindness has not been reported previously. The histopathological findings in one of our cases indicating renal dysplasia and retinal aplasia seem to present an unusual combination of two genetically determined abnormalities.

### Summary

Two siblings, a boy and a girl, with congenital blindness died at 8 and 9 years of age from renal failure. Autopsy was performed in one case. The kidneys showed evidence of dysplasia and of pyelonephritis. Earlier suggestions that renal dysplasia might be the primary cause in cases of chronic pyelonephritis in children are supported. Examination of the eyes indicated a congenital defect, retinal aplasia.

### References

1. ALSTRÖM, C. H. and OLSON, O.: Heredo-retinopathia congenitalis. *Hereditas*, 43, 1957, p. 178.
2. BODECHTEL, G. and ERBSLÖH, F.: Handbuch der speziellen pathologischen Anatomie und Histologie. XIII, B. 2. 1958. p. 1419. Springer Verlag, Berlin.
3. ERICSSON, N. O. and IVEMARK, B. I.: Renal dysplasia and pyelonephritis in infants and children. I. *Arch Path*, 66: 255, 1958.
4. — Renal dysplasia and pyelonephritis in infants and children. II. *Arch Path*, 66: 264, 1958.
5. FROLICH, TH.: To tilfælde av hereditær, familier, kongenital (?) nefrit. *Norsk Mag Læge*, 65: 905, 1904.
6. GOLDBLOOM, R. B., FRASER, F. C., WAUGH, D., ARONOVITCH, M. and WIGLESWORTH, F. W.: Hereditary renal disease associated with nerve deafness and ocular lesions. *Pediatrics*, 20: 421, 1957.
7. HAWKINS, C. F. and SMITH, O. E.: Renal dysplasia in a family with multiple hereditary abnormalities including iliac horns. *Lancet*, 1: 803, 1950.
8. IVEMARK, B. I., OLDFELDT, V. and ZETTERSTRÖM, R.: Familial dysplasia of kidneys, liver and pancreas. A probably genetically determined syndrome. *Acta Pædiat*, 48: 1, 1959.
9. KEELER, C. E.: Rodless retina, an opthalmic mutation in the house mouse, *mus musculus*. *J Exp Zool*, 46: 355, 1927.
10. KEELER, C. E.: Normal and rodless retinae of the house mouse with respect to the electromotive force generated through

- stimulation by light. *Proc Nat Acad Sc*, 14: 477, 1928.
11. MARSHALL, A. G.: The persistence of foetal structures in pyelonephritic kidneys. *Brit J Surg*, 41: 38, 1953.
  12. MITCHELL, A. G.: Nephrosclerosis (Chronic interstitial nephritis) in childhood. *Amer J Dis Child*, 40: 101, 1930.
  13. RYERSBACH, G. C. and BUTLER, A.: Congenital hereditary hematuria. *New Engl J Med*, 251: 377, 1954.
  14. SCHAPPERT-KIMMISER, J., HENKES, H. E. and VAN DEN BOSCH, J.: Amaurosis congenita. *Arch Ophtal*, 61: 211, 1955.
  15. SOHAR, E.: A heredo-familial syndrome characterized by renal disease, inner ear deafness and ocular changes. *Harefuah*, 47: 161, 1954. Cit. Goldbloom et al., *Pediatrics*, 20: 421, 1957.
  16. SORSBY, A. and WILLIAMS, C. E.: Retinal aplasia as a clinical entity. *Brit Med J*, 169: 293, 1960.
  17. WOLFF, E.: A Pathology of the Eye. p. 165. Lewis and Co. LTD., London 1951.

Received May 15, 1960

Accepted June 15, 1960

Pediatric Clinic  
Rikshospitalet  
Oslo  
Norway

From

## Polycystic Kidneys in Newborns, Infants and Children

### A Clinical and Pathological Study

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#### Introduction

Cystic kidneys occur in adults as well as in children of all ages. While the adult variety of the disorder usually is inherited dominantly, it may also occur without manifest heredity.

Cystic kidneys in children of various ages have been the subject of very divergent opinions, both as regards their relationship with the adult form and with respect to their heredity. Most authors still treat cystic kidneys as a disease entity where the degree of parenchymal destruction is the sole factor determining the patient's chances of surviving.

As early as 1902, Küster showed that the age distribution of 239 subjects with the disorder had two peaks; one at the time of birth (59 stillborn babies) and another between the ages of 30 to 60 years (Fig. 1). Later, other authors have confirmed this distribution (9, 23). The consensus of all workers who in recent years have studied the age distribution of cystic kidney cases is that the disorder is encountered in children of all ages, albeit less frequently in infants and young children. Yet no

preponderance is found among newborns and infants in any of the published family trees showing the inheritances of the dominant adult form; actually, as far as we know, it has not been diagnosed in any child less than 2 years of age in these families and some workers believe that cystic kidneys in newborns and children are recessively inherited (6, 23).

Studying cystic kidneys in newborns (2 cases), on the one hand, and in older children and adults on the other, Lambert (18) demonstrated that the cystic kidneys in the two groups differed histologically. The existence of such a difference has subsequently been verified (4). Continued research on the subject has revealed not only that there exist two forms of the condition—the neonatal and the adult—but also that the former occurs in two or more histologically discrete varieties (22, 25, 30).

Many problems posed by newborns and infants with cystic kidneys—their clinical manifestations, heredity and prognosis as well as the question of their relationship to cystic kidneys in older children and adults—will hardly be solved unless

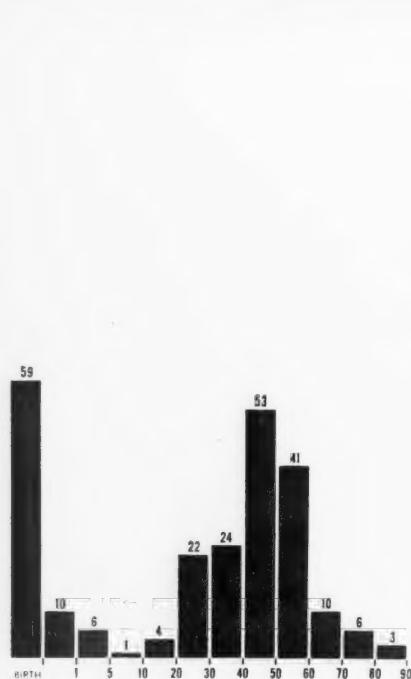


Fig. 1. Age distribution according to Küster.

one finds means of morphologically distinguishing the different types. Unfortunately most monographs on cystic kidneys have in common the weakness that the material has been imperfectly analyzed from histological points of view and hence clinical and genetic peculiarities are obscured or difficult to interpret. Dalgaard (6), for example, drew conclusions about cystic kidneys in newborns and children despite the fact that in his series of 40 cases the kidneys were not examined microscopically.

#### Material (Including the Criteria of Diagnosis)

According to our concept cystic kidneys are characterized by bilateral diffuse scatter-

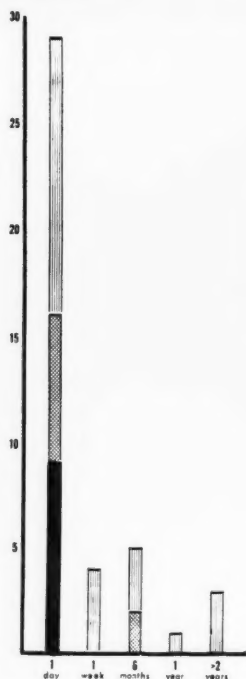


Fig. 2. Age distribution in present material. ■ Group I, □ Group II, □ Group III-IV.

ing in the parenchyma of a large number of cysts of varying sizes. The microscopical appearances range from those where the cysts are entirely dominant and look like the meshes in a coarse network supplanting the normal renal parenchyma, to those where the cysts are small and interspersed by normal-looking parenchyma. Grossly the kidneys may exhibit the usual kidney-shape or may be greatly deformed; they may be anything from hypoplastic to enormously hyperplastic. We have excluded from the series all cases of unilateral cystic kidney where the contralateral organ was normal, as well as all cases of circumscribed, unilocular or multilocular cystic formations.

Potter (22) described familial occurrence of large spongy polycystic kidneys in newborns. The familial occurrence of this type was also observed by one of us (20). This prompted the idea of analyzing the clinical





Fig. 3. Polycystic kidneys in situ. Group I.



Fig. 4. Polycystic kidney, Group I.  $\times 0.6$ .

manifestations and heredity of such neonatal cystic kidneys and led to the collection of a 10 year series from the whole of Sweden.

After perusal of the Annual Reports for the decade 1945-1954 from all Swedish hospitals, relevant extracts from the case records were collected regarding 27 cases which complied with our criteria. To these cases were added 14 similar ones observed from 1945 through 1958 in Gothenburg (Table 2). In 26 of these cases it proved feasible to carry out a thorough histological examination. In addition the existence of cystic kidneys in a living child (7B, sibling of child 7A) was established by clinical diagnosis.

Fig. 2 shows the age classification of our series of 41 cases.

In Gothenburg, where all autopsies included a search for cystic kidneys, 14 cases were encountered among the 3200 autopsies performed at the children hospitals and obstetrical departments from 1945 through 1958. This is equivalent to approximately 1 case per 230 autopsies.

We have divided into four groups the histologically examined cases in our series. The fourth group contained only four cases

of kidneys which in no way resembled each other in appearance. It should be noted that the classification was made exclusively on morphological grounds and consequently should not be made a basis for pathogenetic conclusions. Nor does it make claims to be final. Groups I, II and III have nevertheless a distinct appearance both grossly and microscopically.

### Group I

These kidneys usually weigh around 400 g per pair and are considerably oversized but have retained the characteristic shape. The surface is smooth and copious masses of tiny cysts are dimly visible through it. On the surface of the section, which has a spongy appearance, one discerns numerous cystic spaces of round or slot-like shapes situated close together in a radial arrangement extending all the way from the capsule to the renal pelvis. The renal pelvis is gracile, the ureter slender and thin-walled (Fig. 4).

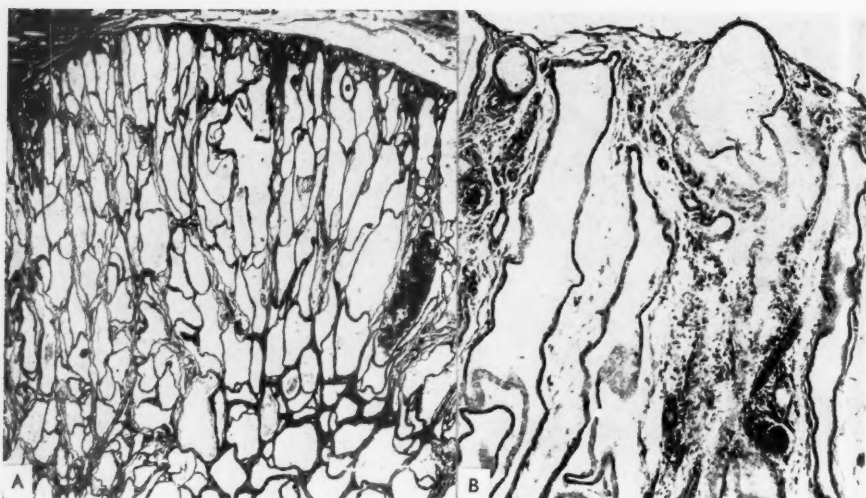


Fig. 5. Polycystic kidney. Group I. A.  $\times 10$ . B.  $\times 120$ .

Microscopical examination discloses in a narrow marginal zone next to the cortex, remotely spaced and occasionally incompletely developed glomeruli with not-dilated Bowman's capsules and a few often poorly developed tubular structures. The rest of the parenchyma is entirely occupied by the aforementioned round or elongated cystic spaces which are lined by a regular, cubical epithelium. In the epithelium the nuclei are rather large, the intercellular borders indistinct. (Nothing suggests that the cells are of tubular origin.) Down towards the renal hilus the cysts become more irregular, obtunded, larger. In juxtacapsular areas very small amounts of loose, collagen-poor tissue is found between the cysts, but as the renal hilus is approached the interstitial tissue becomes more plentiful and richer in collagen fibres. Morphologically fully developed nephrons with differentiated distal and proximal convoluted tubules are

seldom encountered in cystic kidneys belonging to Group I (Fig. 5).

This group is the equivalent of what Sanchez-Lucas termed spongy kidneys (*rein d'éponge*).

In three cases belonging to this group histological examination of the liver revealed a marked proliferation of the finer bile ducts some of which were dilated and pronounced periportal fibrosis (Fig. 7). Another four cases were found to have cysts in the liver and one of them in the pancreas too, but unfortunately the organs were not examined histologically. In two cases where pancreas were examined microscopically a marked fibrosis of the parenchyma, dilatation of the ducts and periductular fibrosis were found (Fig. 8).

#### *Clinical Data*

Group I comprised 2 boys and 7 girls. Six of the children were observed by ourselves immediately or shortly after parturition.

### *Pregnancy and Parturition*

Six of the children were born before the 38th week, the remaining three in the 39th–40th week. Four of the children were delivered by breech presentation. From 2 to 4 % of normal births are breech presentations (11).

Three parturitions observed by us were associated with hypamnion. In the other cases the amniotic fluid was reportedly normal but nothing was recorded as to its volume. Many hypotheses have been presented for the mode of production of amniotic fluid, particularly with reference to the part played by the urine (16, 31). Several authors have also noted hypamnion in urinary tract malformations and in renal agenesis (7, 27). Hypamnion in cases of cystic kidneys has also been observed by Singer (28). The existence of dry labour in the neonatal type of cystic kidneys suggests that such kidneys secrete no urine.

One of the mothers had jaundice during late pregnancy, another was anemic and the rest seemed in the best of health.

### *Symptoms in the Child*

The children in this group were asphyctic, their respiration irregular or shallow and breathing difficulties were dominant throughout the survival period. No child survived more than 4 hours, one died immediately after birth, none was still-born.

The most striking feature seen when these children are examined is the greatly distended abdomen, and when it is palpated the kidneys are invariably found to be markedly enlarged (Fig. 3).

Potter described a specific facial ex-



Fig. 6. "Potters face" in case of polycystic kidney. Group I.

pression in children with renal agenesis (deep eye furrows, snub-nose, micrognathia, large flabby ears placed low on the head). But the same thing occurs in other renal malformations too. The 6 children we had the opportunity to observe personally all had a typical "Potter face" (Fig. 6).

Other malformations, except for cysts in other parenchymatous organs, have been observed in only one case, namely stenosis of the sigmoid flexure.

Why these children die so soon after birth is a question few authors have discussed and the general belief seems to be that death occurred as a result of uremia. The mere fact that children with renal agenesis frequently survive for several days with a slowly rising nonprotein nitrogen levels effectively disproves the view that renal insufficiency is the cause of death. Acid-base disequilibrium due to urine retention could presumably be a contributory cause of death. However,

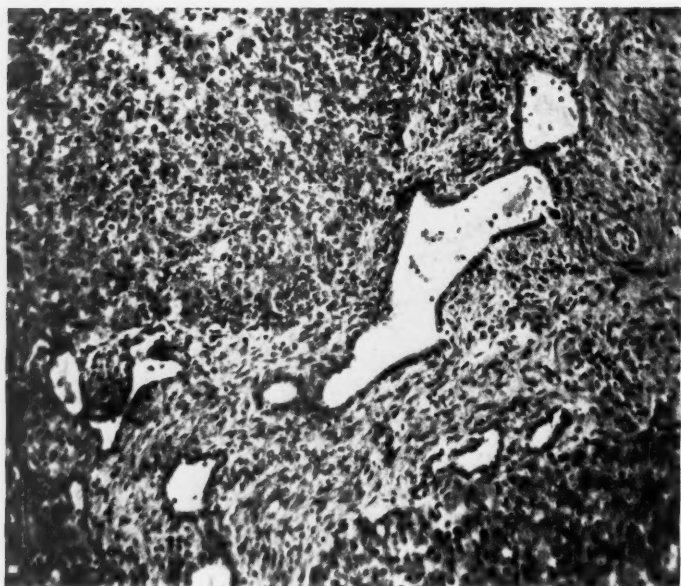


Fig. 7. Duct dilatation and fibrosis in liver. (Group I.)  $\times 135$ .

such a disequilibrium would only arise if the placental barrier was damaged. In one of our cases (No. 5) a complete blood analysis was done immediately before death. The Na, K,  $\text{Co}_2$  etc. levels were quite normal for the age and N.P.N. was 48 mg %. Dalgaard (6) pointed out that these children presumably die from other causes than their kidney malformation (premature birth, pulmonary atelectasis, other malformations incompatible with life).

Cystic kidneys of Group I seem to show something of a hereditary trait. Six of our 9 cases occurred in three families. (This will be further commented upon).

### Group II

Most of these kidneys are considerably enlarged, up to 400 g per pair. While the

typical renal shape usually is preserved, occasional organs may be more or less deformed by large cysts (Fig. 9).

Microscopically the cysts exhibit a cuboidal or simple squamous and regular epithelium. The cysts are surrounded by large amounts of fairly loose and moderately collagen-rich connective tissue. The cysts in some kidneys occupy the greater part of the parenchyma and one finds only small residual portions of more normal parenchyma with a few apparently underdeveloped glomeruli and irregular tubular structures next to the cortex. In other kidneys those areas which contain cysts and much connective tissues are mixed up with patches of seemingly unchanged renal tissue where there are histologically fully normal nephrons with identifiable proximal and distal convoluted tubules (Fig. 10).

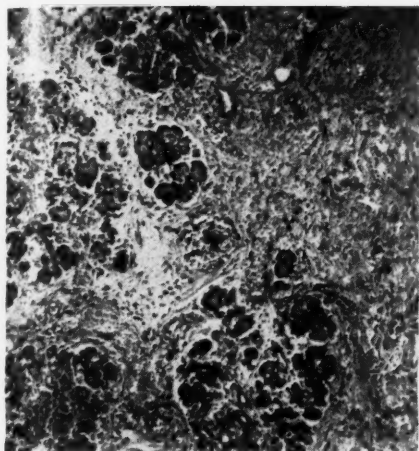
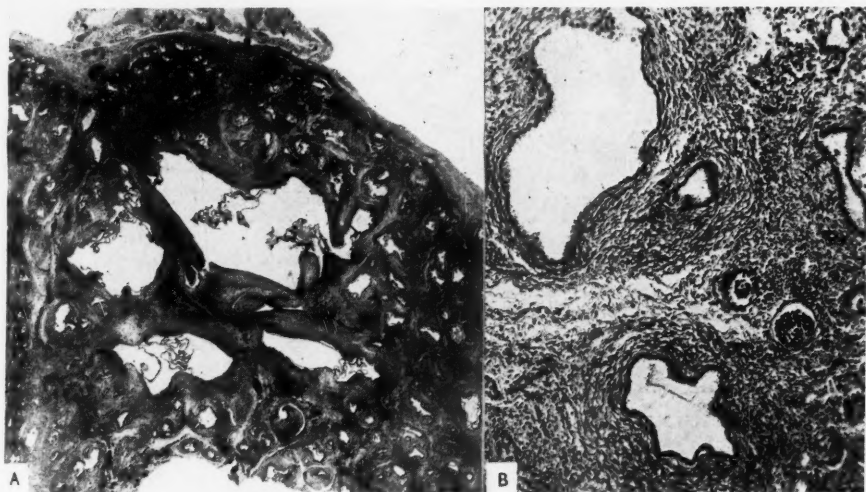


Fig. 8. Fibrosis of pancreas. (Group I.)

Fig. 9. Polycystic kidney. Group II.  $\times 0.8$ .Fig. 10. Polycystic kidney. Group II. A.  $\times 10$ . B.  $\times 150$ .

This kidney is indistinguishable from the cystic kidney rich in connective tissue described by Potter. It might be identical with the kidney Staemmler described as being morphologically equivalent to the adult type of cystic kidney.

Malformations in other organs have not been observed. In the few cases where liver and pancreas were examined fibrosis of the same kind as in Group I could not be found.

### *Clinical Data*

Included in this group are 10 children (5 boys and 5 girls) from 8 families. One of the children (No. 7B) is at present 3 years of age with clinical signs (including X-ray) of cystic kidneys.

### *Pregnancy and Parturition*

Six of the children were born in the 38th week, one in the 36th and one in the 39th. For the two others nothing is known about the duration of pregnancy.

Specific information about the amount of amniotic fluid was available for two cases only; it was normal in amount.

Six of the children were born in breech presentation, 3 in vertex presentation and in 1 case the mode of presentation was not recorded.

### *Symptoms in the Child*

Apart from Case 7B, we personally observed only one of the children in this group. Two of the children (Cases 12 and 13) survived the neonatal period and died with renal insufficiency at the ages of 1.5 and 4 months, respectively. The remaining six children died within a few hours of birth.

In this group the only other malformation we observed was a case of occipital meningocele and a contributory cause of death was intracranial hemorrhage in Case 11 and hemolytic anemia of newborns in Case 9.

Also in children with this type of cystic kidneys the condition appears to have a familial incidence, in two families two siblings were affected.

### **Group III**

Kidneys in this group are hypoplastic and have cysts of varying size. Occasion-

ally they become as big as plums and then they deform the organ. Although the parenchyma always is hypoplastic, the kidney may have a considerable total weight owing to the presence of very large solitary cysts. The uriniferous passages in some cases exhibit severe malformations, for example folded or dilated ureters or absence of ureters (Fig. 11).

Microscopically the cysts are lined with a squamous or cubical epithelium and surrounded by fairly much connective tissue. As a rule one sees only small areas in which there are a few mostly immature glomeruli and, in association with these, occasional tubular structures (Fig. 11). In some cases cartilage was found in the parenchyma.

Included among the hypoplasias by Bell (2), this type of kidney is identical with Staemmler's third type. It resembles in many respects the "dysplastic" cystic kidneys in children described by Schwartz (26). (Cf. also Parkulainen, and Ericsson *et al.*)

### *Clinical Data*

Four boys, two of them uniovular twins, from three families belonged to this group.

### *Pregnancy and Parturition*

One of the children was born in the 39th, the others before the 36th week.

No information regarding the amount of amniotic fluid is available in any of the cases. Micturition definitely occurred in the twins who died after 4 and 7 days. The other children died within one hour.

The pregnancies without exception appeared normal and the children were all born in vertex presentation.





Fig. 11. Polycystic kidney, Group III.  $\times 6$ .

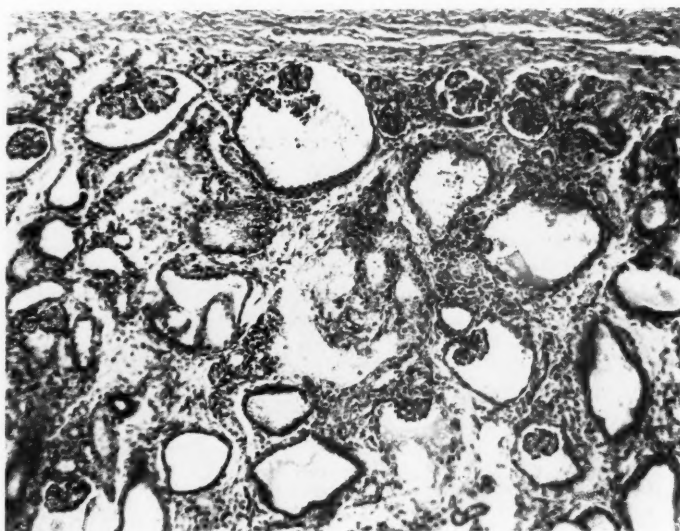


Fig. 12. Glomerular type of polycystic kidney.  $\times 150$ .



*Symptoms in the Child*

One of the twins (No. 16A) had hydro-nephrosis on the right side in addition to cystic kidneys. Moreover, urinary tract malformations were observed in the other children of this group.

In the first of the twins (No. 16A) the existence of renal lesions was not suspected. But as cystic kidneys were found at autopsy, an N.P.N. analysis was made on the second shortly before death, the value being 89 mg %. The first twin died after a convulsion, the second in conjunction with a cyanotic attack. Both twins suffered from respiratory difficulties and had repeated attacks of cyanosis.

**Group IV**

To this group were assigned four cases that could not be classified under any other group on account of the histological picture. In two cases the cysts microscopically consisted of dilated glomeruli, the kidneys accordingly being equivalent to Potter's glomerular type of cystic kidney (Fig. 12). In one of these two cases no information was available regarding the size and gross appearance of the kidneys, in the other they were unequally large and weighed 8 and 260 g respectively. In one of the other two cases the kidneys were small and had some cysts in the cortex. Microscopical examination disclosed a cortex and medulla with, on the whole, normal histology, large numbers of moderately dilated tubular structures being visible along the cortico-medullary border and in the cortex.

*Clinical Data*

This group comprised one boy and three girls. The mothers had apparently normal

pregnancies. One of the children with glomerular cysts died a few hours after parturition, the other at the age of five years of renal insufficiency. The latter child had amaurosis and a cerebral malformation.

**Cystic kidney cases not examined histologically by us**

Histological examination of the kidneys from 15 children could not be carried out because microscopical slides were not available. However, all the children concerned had been autopsied at the local hospital, so the diagnosis of cystic kidneys had at any rate been made by gross inspection. In those cases where histological information was available we dared not use it as a basis for classifying the cases into our groups.

**Discussion***Morphological remarks*

Neonatal and infantile polycystic kidneys exhibit a great variety of gross and microscopic pictures. Yet few authors have attempted to distinguish between morphologically different types. Sanchez-Lucas recognized two distinct varieties of neonatal cystic kidneys, namely one type where the kidney usually is of normal size and has large cysts—similar to our Group III—and another type where the kidney is hyperplastic, usually enormously enlarged, and has small cysts and a spongy consistence (rein d'éponge)—identical with our Group I.

Potter (22) distinguished two different main types: one where the amount of connective tissue is normal and another

No.

Gro

1A

1B

2A

2B

3

4

5A

5B

6

Gro

7A

7B

8A

8B

9

10

11

12

13

14

Gro

15

16

17A

17B

Gro

18

19

20

21

N.

22

23

24

25

26

27

28

29

TABLE 1.

No.	Sex	Birth weight g	Weeks of gest.	Presentation Breech = B Vertex = V Caesarian section = C.S.	Survival time	Kidney weight Right Left	Other malformations	No. of sibs	Affected sibs
<i>Group I</i>									
1 A	♀	3.190	37	B	3 h	250	Liver cysts	3	1
1 B	♀	2.500	36	B	1 h	250	—	3	1
2 A	♀	3.690	38	B	1 h	450	Liver cysts, pancreatic cysts	6	1
2 B	♀	4.200	35	V	Dead at part.	Very enlarged	Liver cysts	6	1
3	♂	3.600	37	V	4 h	195 210	—	1	0
4	♀	2.500	36	B	1 h	250	—	1	0
5 A	♀	3.290	39	V	1 h	460	Fibrosis and duct dilatation of liver and pancreas	3	1
5 B	♂	2.900	39	B	1 h	330	—	3	1
6	♀	3.360	40	V	2 h	105 108	— and sigm. stenosis	1	0
<i>Group II</i>									
7 A	♀	2.410	35	B	4 h	? ?	—	1	1
7 B	♀				Alive		—	1	1
8 A	♀	2.560	34	V	4 h	Large	—	1	1
8 B	♀	3.310	39	B	1 h	Large	—	1	1
9	♀	2.700	35	V	5 min.	? ?	Erythroblastosis	1	0
10	♀	3.220	38	B	1 min.	400	—	2	0
11	♀	2.420	37	B	2 h	Large	—	0	0
12	♀	2.770	?	?	3 m	100 100	—	1	0
13	♀	3.350	?	B	4 m	Large	—	1	0
14	♀	2.850	36	V	7 h	80 80	Meningocele + polydactyli	4	0
<i>Group III</i>									
15	♂	2.560	34	V	1 h	0 140	—	4	0
16	♂	3.570	39	V	Dead at part.	Large	—	0	0
17 A	♂	2.600	36	V	7 d	Norm. Small	—	2	1
17 B	♂	2.190	36	V	4 d	Small	—	2	1
<i>Group IV</i>									
18	♀	2.325	38	B	2 h	8 260	—	0	0
19	♀	4.100	40	?	5 y	? ?	Cerebr. malfor- mation	1	1
20	♀	3.000	38	V	1 h	400	—	2	0
21	♂	2.200	34	V	2 m	Small	—	2	0
<i>Not histologically examined</i>									
22	♀	2.740	36	B	1 h	Large	—	1	0
23	♀	2.250	34	V	1/2 h	? ?	—	1	0
24	♀	4.500	41	C.S.	1 min.	Large	Mongol	0	0
25	♀	2.720	35	B	1 h	? ?	—	0	0
26	♀	1.600	30	B	1 min.	Small	Hydrourerter	2	0
27	♀	2.540	35	B	Dead at part.	Very enlarged	—	2	0
28	♂	1.550	37	B	3 h	0 Small	Mongol	3	0
29	♀	1.880	31	V	Dead at part.	650	Encephalocele	0	0

(Table 1 cont.)

No.	Sex	Birth weight g	Weeks of gest.	Presentation	Survival time	Kidney weight		Other malformations	No. of sibs	Affected sibs
				Breech = B Vertex = V Caesarian section = C.S.		Right	Left			
30	♂	2.500	?	V	1 h		Large	—	1	0
31	♂	2.340	37	?	5 d		Large	Hydronephrosis	1	0
32	♀	3.520	40	V	3 m	Norm. Large		Hydronephrosis	1	0
33	♂	2.050	32	V	2 d		? ?	Hydronephrosis, Stricture of ureter	0	0
34	♂	3.590	?	?	1 y		Small	Hydronephrosis	2	0
35	♂	3.170	?	?	1 y		Small	—	1	0
36	♂	3.660	?	?	2 y		Large	Hydrocephalus	2	0

where it is greater than normal. In the first category the cysts may consist of either dilated and elongated tubules (our Group I) or—but never *and*—dilated glomeruli (similar to cases in our Group IV). In Potter's second category the cysts are of tubular origin (our Group II).

Using a somewhat different basis for classification, Staemmler (30) distinguished three types of neonatal or infantile cystic kidneys. The first type is characterized by a very large kidney closely resembling that seen in the adult form of the disease (our Group II); the second type exhibits a moderately enlarged kidney of spongy consistence (similar to Sanchez-Lucas' rein d'éponge and our Group I); the third type is a hypoplastic kidney of irregular configuration (included among the hypoplasias by Bell and our Group III).

The most probable reason why different principles for classification have been employed would seem simply to be that the material available to the various investigators frequently are too small. In most papers dealing with "large" material the histology has not been fully described.

#### *Cysts in other organs*

As many authors have pointed out (for example, see Potter), subjects with cystic kidneys frequently have cysts in other organs as well, most often in the liver but also in the lungs and pancreas.

In our material the findings of cysts in other organs are strictly limited to cases belonging to Group I. In this group we have by microscopical examination observed a proliferation of bile ducts and periportal cirrhosis in all examined cases who did not exhibit gross cysts in the liver. These findings are in agreement with Potter's. Moreover, we have observed fibrosis and duct dilatation in pancreas in two cases belonging to Group I.

#### *Pathogenesis*

Numerous papers have been devoted to the formal genesis of cystic kidneys. The problem has been attacked along two main lines. Either the type of the cyst epithelium as it appears under the microscope has been used to trace the origin of the cysts to certain structures, or microdissection and serial sectioning with subsequent reconstruction have been employed in at-

tempts to derive the cysts from the various components of the nephron.

Using the reconstruction method in two cases of neonatal cystic kidneys, Lambert (18) discovered that the cysts were of glomerular, tubular and possibly also collecting tubule origin. He stated that the cystic nephrons invariably were separated from their "secretory tubules".

Bialestock (3) applied the microdissection technique in one case of cystic kidneys from a prematurely born child, finding that most of the cysts were derived from the glomerules and some most likely from the tubules. Also the non-cystic nephrons exhibited changes in the form of short, irregular and thin-walled tubules. Bialestock was unable to confirm Lambert's observation that the cystic nephrons lacked communication with the collecting tubules.

As it is impossible to tell from the morphological observations reported by these two authors what type of cystic kidney they investigated, their cases cannot be fitted into the classification adopted by us. Most likely they were observing quite different types of cystic kidneys. Nevertheless, although they warrant no generalized conclusions regarding the formal genesis of cystic kidneys, their findings do indicate that the cysts may originate from different parts of the nephron.

Different types of cysts can seldom be recognized solely on the basis of histological appearances. The large majority of cysts are lined with either cubical or pavement epithelium lacking characteristic morphological features. The appearance of such epithelium obviously provides no clues as to whether the cyst originates from a particular part of the

nephron. The histological appearance is specific only in those cases where one definitely sees glomerular cysts with glomerular coils in the wall and also in those cases where the cysts are tubular and occur together with cystically dilated tubules and fully normal tubular structures.

Such morphological observations combined with known facts about the normal evolution of the kidney have been the basis for many published theories regarding the genesis of cystic kidneys. As early as 1894 Hildebrand (12) presented the theory, which was later supported by Ribbert (24), that various nephronic components arising from the metanephrogenous blastema receive no communication to the collecting tubules so that urine secreted from the glomeruli is not drained away and accumulates in the nephron which ultimately leads to the formation of a cyst.

Kampmeier (13) expressed the opinion that the first generations of glomeruli formed in the kidney normally degenerate, a process which often can give origin to small residual cystic structures. The causative factor of polycystic kidney might thus be exaggeration of the normally occurring, mild cystic degeneration of embryonic glomeruli (14).

Other authors have drawn attention to the possibility that interstitial inflammation might cause occlusion of the tubules (1).

Cystic kidneys have also been interpreted as a neoplastic abnormality secondary to pathological epithelial proliferation in the tubules (5, 30).

As pointed out by a few authors who have classified various types of cystic kidneys, there is no reason to believe that all types necessarily must have either the

same etiology or the same formal genesis (22, 30). Potter studied 50 cystic kidneys from newborns and infants without finding anything in support of either of the two most common theories regarding the etiology of the abnormality. She pointed out that abnormal development of various parts of the nephron may produce aberrations of a type that would enable cysts to be formed. As other organs too, often exhibit changes with formation of cysts, the process seems to be one that is not confined exclusively to the kidneys. This applies particularly to cystic kidneys of Group I in our classification.

### *Etiology*

Occasional cases have been described in which the mother of a child with cystic kidneys had german measles during pregnancy (19). In our series only two of 36 mothers had been exposed to german measles during pregnancy (in the 2nd and 4th month respectively) without clinical signs of infection. One mother had erythema nodosum in the 2nd month, the others were all healthy during the first half of pregnancy. It can safely be assumed that any viral disease occurring during pregnancy would have been noted, at least in the mothers of children born later than 1950, because after that year viral diseases in pregnant women have attracted particular interest in this country.

Although many authors have postulated that cystic kidneys in infants are inherited recessively (6, 29, 33), no study seems to have been carried out on a morphologically uniform material. The present phase of our research concerning cystic kidneys includes superficial genealogical analysis.

The preliminary investigation—consisting of simple interviews with the parents—disclosed no evidence of cystic kidneys occurring in ancestors or relatives, nor did it provide proof of any consanguinous marriages.

However, when in the Group I type malformation, where the children are unviable and die within a few hours after birth, there is a familial occurrence, a recessive heredity must be assumed. Our Group I includes 9 cases of polycystic kidneys among 21 sibs. When the figures are treated for the selection bias in principle according to Weinberg (32) the corrected figures will be 6 affected among 27 sibs (Table 2).

Thus, the affected comprise 22.2 % of all sibs, corresponding to the expected 25 % is as good as can reasonably be demanded. It has consequently been shown that the manifestation frequency in our Group I agrees with the assumption of the heredity of a single recessive gene. The risk for a family with this recessive gene of having an abnormal child is accordingly one in four. The prognosis for the affected child is grave. For siblings who do not exhibit the clinical symptoms described in the foregoing and do survive the first day or days the risk of their having cystic kidneys is nil but, in accordance with the above assumptions, two thirds of the healthy children will carry the gene.

In practice this implies that when a case of cystic kidneys of Group I has occurred the prognosis for subsequent children is reasonably favourable and so the parents, provided they are aware of and understand the risks involved, need not be cautioned against having any more children. Notably, however, the healthy chil-

TABLE 2. Group I. Correction for the selection bias according to Weinberg.

Conductors	No. of children	Affected (A)	Unaffected (U)	A (A-1)	AU
1 A, B	4	2	2	2	4
2 A, B	7	2	5	2	10
3	2	1	1	0	1
4	2	1	1	0	1
5 A, B	4	2	2	2	4
6	2	1	1	0	1
Total	21	9	12	6	21

dren should be strongly discouraged from contracting consanguinous marriages.

Group II includes 10 cases among 19 sibs. After correction as above the affected comprise 18.1 %. As, in this group, the kidneys have a more or less intact parenchyma enabling the subjects in some cases to live for weeks or years, and we have investigated only few sibs, we cannot use this figure as a proof of recessive heredity. Thus the heredity is not fully understood, but the familial occurrence is documented and the affected child can sometimes live for quite a long time.

This must be considered in discussing additional children with the parents.

### Summary

Twenty-eight cases of polycystic kidneys in newborn, infants and children have been investigated clinically and histopathologically. Three more or less distinct morphological types of polycystic kidneys are described and in addition single cases of deviating appearance. The clinical and hereditary aspects are discussed. In Group I (spongy kidney) a single gene recessive heredity has been proved.

### References

1. ARNOLD, J.: Über angeborene einseitige Nierenschrumpfung mit Cystenbildung. *Beitr Path Anat*, 8: 21, 1890.
2. BELL, E. T.: Renal Diseases. Philadelphia 1947.
3. BIALESTOCK, D.: The morphogenesis of renal cysts in the stillborn studied by microdissection technique. *J Path Bact*, 71: 51, 1956.
4. BRUCKER, N. S. and PATTON, J. F.: Cystic disease of the kidneys. *Amer J Med*, 18: 207, 1955.
5. BUGIDI, V. and SEVERI, A.: Contributo alla patogenesi delle cisti renali. *Sperimentale*, 46: 1, 1880.
6. DRAGAARD, O. Z.: Bilateral Polycystic Disease of the Kidney. Diss. Munksgaard, Copenhagen 1957.
7. DAVIS, E. and POTTER, E.: Congenital malformations and obstetrics. *Pediatrics*, 19: 719, 1957.
8. ERICSSON, N. O. and IVERMARK, B. Z.: Renal dysplasia and pyelonephritis in infants and children. *Arch Path*, 66: 264, 1958.
9. FERGUSON, J. D.: Observations on familial polycystic disease of the kidney. *Proc Roy Soc Med*, 42: 806, 1949.
10. — Observations on familial polycystic disease of the kidney. *Brit J Urol*, 21: 241, 1949.
11. HEDBERG, E.: Breech deliveries. *Acta Obstet Gynec Scand*, 36: 356, 1957.
12. HILDEBRAND: Weiterer Beitrag zur pathol. Anatomie der Nierengeschwülste. *Arch klin Chir*, 48: 343, 1894.
13. KAMPMEIER, O. F.: A hitherto unrecognized mode of origin of congenital cysts. *Surg Gynec Obstet*, 36: 208, 1923.

14. KAMPMEIER, O. F. and McKENNA, C. M.: A consideration of the development of polycystic kidney. *J Urol*, 32: 37, 1934.
15. KINNUNEN, O.: Fostervattnets uppkomst. *Nord Med*, 45: 56, 1951.
16. KINNUNEN, O. and PAALANEN, A.: Fostrets njurfunktion. *Nord Med*, 47: 19, 1952.
17. KÜSTER, E.: Die chirurgischen Krankheiten der Niere. *Deutsch Chir*, 52 B: 512, 1898-1902.
18. LAMBERG, P.: Polycystic disease of kidney. *Arch Path*, 44: 34, 1947.
19. LIPTON, E. L. and SCORDAMAGLIA, L. J.: Congenital unilateral multicystic kidney associated with maternal rubella. *J Pediat*, 50: 730, 1957.
20. OLOW, I.: Congenitala cystnjurar. *Nord Med*, 54: 1461, 1955.
21. PARKULAINEN, K. V., HJELT, L. and SIROLA, K.: Congenital multicystic dysplasia of the kidney. *Acta Chir Scand*, Suppl 244, 1959.
22. POTTER, E.: Pathology of the Fetus and the Newborn. Chicago 1952.
23. RALL, J. E. and ODEL, H. M.: Congenital polycystic disease of the kidney: review of the literature and data on 207 cases. *Amer J Med Sc*, 218: 399, 1949.
24. RIBBERT, H.: Über die Entstehung der Cystenniere. *Verhandl Deutsch Path Gesellsch*, 2: 187, 1900.
25. SANCHEZ-LUCAS, J. G.: Morphogénèse d'un rein polykystique. *Sém Hop Paris*, 27: 2793, 1951.
26. SCHWARTZ, J.: Unusual unilateral multicystic kidney in infants. *J Urol*, 35: 259, 1936.
27. SELBY, G. W. and PARMELEE, A.: Bilateral renal agenesis and oligohydramnios. *J Pediat*, 48: 70, 1956.
28. SINGER, H. A.: Kongenitale Cystennieren beim Neugeborenen. *Surg Gynec Obstet*, 38: 768, 1924.
29. SORSBY, I.: Clinical Genetics. Butterworth, London 1953.
30. STAEMMLER, M. in KAUFMANN, E. and STAEMMLER, M.: Lehrbuch der speziellen pathologischen Anatomie. Berlin 1957.
31. THIEESTEIN, S., COLEMAN, F. and TANNER, F.: Kidney function in the fetus. *Amer J Obstet*, 56: 1178, 1948.
32. WEINBERG, W.: Auslesewirkungen bei biologisch-statistischen Problemen. *Arch Rass & Ges Biol*, 417, 1913.
33. WERNER, M.: Erbbiologie und Erbpathologie des Harnapparates. Handb. der Erb. biol. des Menschen. Berlin 1944.

Submitted Aug. 2, 1960

Accepted Sept. 28, 1960

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## SUMMARY OF SUPPLEMENT

### Studies of Hypothyroidism in Children

by HENNING ANDERSEN

(Supplement 125)

In the *introduction*, *hypothyroidism* in children is defined as any hypofunction of the thyroid gland which affects the growth and development of the child. The designation is used to describe the clinical picture throughout the whole course of childhood, instead of such names as congenital and acquired myxoedema, cretinism, etc. *Athyrosis* is the most severe degree of hypothyroidism.

The work consisted in the main of examining a series of hypothyroid children by means of tests with radioactive iodine, supplemented by other studies, in the first instance examinations of the bones and teeth. The aim was, first to arrive at as early and sure a diagnosis as possible, and secondly to try and sort the hypothyroid children into as uniform groups as possible, according to type and degree of thyroid defect, with the intention of arriving at an improved evaluation of the progress, the effect of treatment, etc.

*Chapter I* gives the history of hypothyroidism and the various views on its etiology. The literature on the mental prognosis for these children is also reviewed here, this being the most important and most discussed clinical problem.

*Chapter II* reviews studies of the uptake

of radioactive iodine ( $I^{131}$ ) in euthyroid children. The author's own procedure is described. (Minimal, 0.25-2  $\mu$ c., doses of  $I^{131}$  have been used, far less than in most other studies.) In 44 euthyroid patients aged 8 months to 17 years, whose symptoms had suggested hypothyroidism, the mean percentage uptake of  $I^{131}$  was found to be  $43.5 \pm 1.5$  after 24 hours, with a standard deviation of 9.7.

*Chapter III* discusses previous studies, including those by the author, on the uptake of  $I^{131}$  in hypothyroid patients. The author's material comprises all those children with primary (thyrogenic) hypothyroidism, examined by means of the above-mentioned technique at Queen Louise's Children's Hospital during the last five years. Cases of secondary (hypopituitary) hypothyroidism are not included in the present material. Three groups could be distinguished:

Group I: 24 children with very reduced or no uptake of  $I^{131}$  over the thyroid gland.

Group II: 14 children with exclusively ectopic, sublingual  $I^{131}$  uptake.

Group III: 18 children with hypothyroidism and goitre.

This distribution deviates considerably

from what is found in other series. The reasons for this are discussed under the separate groups.

*Group I* comprises patients with athyrosis and hypoplasia of the thyroid gland. In these patients, the uptake of  $I^{131}$  lies far below that of euthyroid subjects. Quite good correlation is found between the amount of radioactivity measured over the thyroid gland, and the age at onset of symptoms.

As far as the mental prognosis is concerned, the results suggest that if athyroid children receive vigorous and regular treatment prior to the fourth month of life, it would seem that at least some of them may achieve the level of normal intelligence. If the treatment is instituted after the fourth month, the chances of normal intelligence will be very small. Children with an  $I^{131}$  activity over the thyroid gland of 10% or more are not usually diagnosed until after the first few years of life, and most often their intelligence will be normal.

Among other features of the patients in Group I, it might be mentioned that no association has been demonstrated between the age of the mother and lack of thyroid in the child. With few exceptions, the mothers have not suffered from disease of the thyroid during pregnancy. Pregnancy and labour have generally been normal, although showing a tendency to "postmaturity". In two patients, a positive toxoplasmosis reaction was found in mother and child. The significance of this is not clarified.

The serum cholesterol value was generally elevated in all three groups, while the protein-bound iodine in the serum was reduced, with some few exceptions.

A comparison of birth length, bone development and dental development in athyreotic infants in this material reveals the interesting fact that bone and dental development are prenatally retarded, suggesting intrauterine hypothyroidism, while the birth length is normal or even above normal. The literature gives no exact figures for the birth length in athyreotic children. The factors are discussed which might contribute to the growth of the athyreotic foetus. Attention is drawn to the function of the foetal adrenal cortex.

*Chapter IV* reviews the foetal development of the thyroid gland, particularly its descent from the root of the tongue to its final site in the front of the neck.

*Group II: 14 patients are then described in whom non-palpable, sublingual, ectopic thyroid tissue* was the sole site of uptake of  $I^{131}$ . The author demonstrated this foetal malformation in an infant with "congenital" hypothyroidism of rather slow progression. It was demonstrated elsewhere that a condition such as this could be the cause of some cases of "acquired" hypothyroidism.

As a result of systematic examination of all children without  $I^{131}$  uptake over the normal site of the thyroid gland, the malformation described was found in 14 of a series of 56 hypothyroid patients examined, and may therefore be regarded as not nearly so uncommon as previously assumed. Children of this type present their symptoms somewhat later than the athyreotics, and the prognosis is correspondingly better. In other respects, the group can be associated with the Group I hypoplasias of the thyroid gland.

The sex distribution for Groups I and II

together is that usually given in the literature: twice as many girls as boys. However, if the cases diagnosed before the age of 6 months are considered, the distribution is found to be *equal* between the sexes, while for the cases diagnosed after the age of 6 months, the proportion is still about twice as many girls as boys. Grouping a number of similar series together, the same difference is apparent, and appears to be significant at the 5 per cent level.

*Chapter V* commences with a description of the *normal hormone production of the thyroid gland*, and the disturbances in the production, either as a result of lack of iodine (*endemic cretinism*), or *disturbances of hormone synthesis* on account of congenital enzyme defect and the like.

*Group III*: The author describes 18 patients with *hypothyroidism and goitre*, and attention is drawn for one thing to the pronounced *familial disposition* and to the occurrence of a *large number of deaf-mute children* (about  $\frac{1}{3}$ ) in this group. The reason for this is discussed. At the time of diagnosis the children in Group III are on the average older than the children in Group I. The age distribution is illustrated for all three groups.

The various uptake curves for  $I^{131}$  are discussed, and the histological findings described in some thyroidectomized patients in this group.

*Chapter VI*, on the basis of a review of the literature, discusses *hypothyroidism as a sequela of extrathyroidal enzyme defect*, and *hypothyroidism as a sequela of auto-immunization* (Hashimoto's struma).

*Chapter VII* discusses the relationship between "simple" goitre and *hypothyroidism with goitre*. The author's studies

of 28 euthyroid children with goitre are discussed, with an account of familial incidence, uptake of  $I^{131}$ , content of protein-bound iodine in serum, etc.

*Chapter VIII* discusses the X-ray changes in *hypothyroid children*. The typical epiphysial changes in the long bones, the "dysgeneses", are mentioned, together with the diagnostic significance of these findings. Further, cases are described of a *type of euthyroid child of hypothyroid habitus*, with "*peripheral dysostosis*" of the toes, a condition apparently not previously mentioned in the literature. These children had all been erroneously regarded as hypothyroid.

The X-ray changes in the spine are described, both the localised deformities at the transition between the lumbar and thoracic spine and—more characteristic—the general retardation of the spine. These changes progress more or less parallel with the retardation in the peripheral bone development. A spinal type of "dysgenesis" of the vertebral body was described by the author in 1954. These findings are discussed.

The roentgenological picture of the cranium in *hypothyroidism* is described, with particular reference to the size of the sella turcica, and the significance of this is discussed.

An unusual case is presented of apparent, temporary osteopetrosis in an athyreotic infant, who at the same time showed persisting changes in the enamel of the teeth.

*Chapter IX* reviews the author's studies of the teeth in *hypothyroid children*. It is stressed that the size, number and shape of the teeth are normal, whereas the enamel is hypoplastic and dysgenetic. This

fits in with the times for the appearance of the tooth "anlage", enamel formation and foetal thyroid gland function. *Studies of the frequency of enamel hypoplasias (present in about 80 per cent of the hypothyroid children examined) may be used, for example, to estimate whether hypothyroidism has been present prenatally, as such changes (acquired in utero) are very rarely observed in normal children, or in children with other diseases, and the changes do not disappear during thyroid treatment.*

Dental development is, in general, less sensitive to changes in the thyroid status of the organism than bone development. The same is also the case for other hor-

mones, for example sex hormones and adrenocortical hormones.

*Chapter X* describes previous studies of foetal  $I^{131}$  uptake in man. The conclusion of the author's studies is that the foetus takes up  $I^{131}$  in the thyroid gland—but not in demonstrable amounts at other sites—from about the 12th–14th week of pregnancy, and that the gland appears to be very active.

The *final remarks* discuss in particular the question of early diagnosis, together with the possibilities of genetic prophylaxis and intrauterine treatment of the foetus by treating the mother.

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PROCEEDINGS OF PEDIATRIC SOCIETIES

The Pediatric Society of South Sweden

Meeting, May 8, 1960

**R. Zetterström: Hypoglycemia in Children.**  
(Will be published elsewhere.)

**Per Köhlin: Neurologic Symptoms Among Juvenile Diabetics.**

Of 100 diabetic children born since 1943 examined at Gothenburg's Children's Hospital 8 had on one or more occasions grand mal seizures. These had occurred in connection with hypoglycemia as well as without any certain hypoglycemic symptoms. At the time of the seizures in all 8 children their insulin supply was poorly regulated. They frequently had pronounced glycosuria at the same time as insulin reactions. The disposition to seizures decreased or disappeared when the insulin dose, in spite of the glycosuria, was reduced. Thus, the possibility of overinsulinization as a cause of the seizures appears good. The insulin-induced hypoglycemia can cause a discharge of adrenalin, which in turn leads to increased mobilization of glucose (Somogyi: *Amer J Med*, 26: 1, 1959). It is possible that repeated hypoglycemic attacks produce cerebral lesions which increase the patient's disposition to seizures. Changing the type of insulin did not have any definite influence on the seizures. Peroral preparations have not been employed. EEG examinations, occurrence of convulsions prior to the onset of diabetes and the heredity of the patient yielded no certain evidence for the diagnosis of epilepsy. However, the EEG examinations revealed a high frequency of unspecific abnormality and in two cases paroxysmal activity among the 3 children with convulsions and the 26 other children with labile diabetes. This has been previously observed in older diabetics and their relatives (Izzo: *Diabetes*, 2: 93,

1953; Greenblatt: *New Engl J Med*, 234: 119, 1946; Andersson-Kirstein: *Die Medizinische*, 1: 13, 1959). No focal EEG changes were noticed. The EEG changes may be a manifestation of a posthypoglycemic encephalopathy, may be due to genetic factors or in older patients with focal changes may be a sign of changes in the cerebral blood vessels.

**G. Berglund and B. Lindquist: Generalized Diseases of Bones in Children.** (To be published in *Nord Med*.)

**S. P. Fällström and T. Reinand: Gastric and Duodenal Ulcer in Children.** Clinical and Psychiatric aspects.

A series of 36 children with proven peptic ulcers has been reviewed, and a number of the most recent cases were made the object of special psychiatric-psychologic examinations. Thirty-five children were of school age. There is a marked predominance for boys. The frequency of ulcers seems to have increased in older children. These children often have a strikingly typical ulcer anamnesis. Complications have consisted of hemorrhage in 5 cases and stenosis in 2 cases. The immediate therapeutic results have been satisfactory in most cases. The frequency of recurrence, however, is high. Constitutional and hereditary factors are significant for the occurrence of ulcers in children. Various characteristics connected with the disposition to ulcers are common among these cases. The environmental factors are not equally conspicuous. Neurotic characteristics or emotional disturbances could not be demonstrated to any greater extent in these children.

*Per Selander, Malmö*

## Swedish Pediatric Society

Meeting March 11, 1960

**A. Alvin and R. Tunell: Sternal elevation — an attempt to treat pulmonary atelectasis in newborns**

In newborn infants and particularly pre-matures the fragile and soft thoracic cage may result in pronounced retractions of the lower third of the sternal region, especially in those infants with pulmonary atelectasis. This condition has been compared to that seen in patients with the crushed chest syndrome in whom sternal elevation has proved beneficial. We have therefore treated five newborns with sternal elevation. All had generalized cyanosis, pronounced retractions of the lower third of the sternal region and roentgenological evidence of pulmonary infiltrations.

**Technique.** A double peafil-string suture was placed through the periosteum of the sternum immediately above the ensiform process. The suture was attached to the top of the incubator by a rubber board and the tension so adjusted that the thoracic cage assumed a normal shape. The sternum was kept in this position for 48 hours; in two infants treatment was reinstituted a few days later because of recurrence. In three of the five infants the immediate results were good. These infants had birthweights of 3500, 2170 and 2060 g. In two there was a fall in the respiratory rate and in all three there was a clear clinical improvement after the application of the thoracic elevation. In the other two cases the treatment was without any clinical effect and the children died within 2 days after birth. Post-mortem examination revealed hyaline membranes in both and one had an intracranial haemorrhage as well. It is our intention to continue using sternal elevation in newborn children with the triad of general cyanosis, marked retractions of the lower third of the sternal region and roentgenological signs of pulmonary atelectasis. The management may preferably be restricted to children with a birthweight of more than 2000 g.

**A. J. W. Hagströmer: Paternal age and "fetopathy"**

Advanced paternal age has been shown to be a feature in the etiology of achondroplasia (L. S. Penrose, 1957, and W. Lenz, 1958). A prospective investigation to evaluate the relation between paternal age and "fetopathy in general" has therefore been carried out. The term "fetopathy" as used here includes malformation, stillbirth, neonatal death and/or intracranial hemorrhage. The occurrence of fetopathy at advanced paternal age (i.e. the supposed father over 50 years of age) was compared to that found at low paternal age (i.e. the supposed father under 50 years of age) in a control material. In both groups the mothers were of the same age, parity and social status. The offspring of unmarried mothers were excluded from the study. The incidence of fetopathy in the group of 341 fathers over 50 years of age was found to be 6%. In the offspring of 762 mothers married to men less than 50 years of age the incidence of fetopathy was also found to be 6%. *The influence of paternal age on the incidence of fetopathy registered at birth is thus too slight to be detected in the present quantity of data.*

**A. Alvin: Successful treatment of listeriosis in newborns**

Two cases of listeriosis in newborns are presented. The symptoms were those usual for this age, i.e., acute meningitis and sepsis.

**Case I:** A girl, born 6 weeks before term, weighed 2420 g at birth. During pregnancy the mother had several episodes of bleeding at the time of expected menstruation. From the tenth to the third day prior to delivery she had a septic fever with conjunctivitis, but had no signs of upper respiratory infection, or headache. No treatment was given. On the second day of life the infant became cyanotic and was admitted to the Sachs Children's Hospital. Multiple pinpoint erythematopapular lesions were pre-



sent over the entire body. Treatment with tetracycline was instituted at once and continued for eleven days. Lumbar puncture revealed 580 white cells per mm<sup>3</sup>, of which 560 were mononuclears. The recovery was uneventful. Three weeks post-partum serological examination of the mother revealed an agglutination titer of 1/64 against *Listeria monocytogenes* and a CF titer of 1/160. One month later these values had fallen to 1/32 and 1/15, respectively. The infant was subsequently examined at six months and appeared well.

**Case 2:** A full-term male infant, the product of a normal delivery, was admitted on the second day of life because of cyanosis. Multiple maculopapular lesions were present over the body. Examination of the cerebrospinal fluid revealed 2400 white cells per mm<sup>3</sup> and *Listeria monocytogenes* was isolated on culture both from spinal fluid and from the pharynx. Treatment with chloramphenicol (12 days) and a sulfa preparation (3 weeks) was instituted. The recovery was complete and on examination at six months of age the infant appeared well. The mother from the fifteenth to the sixth day prior to delivery had a slight common cold. Four weeks after delivery the mother's serum showed an agglutination titer against *Listeria* of 1/256 and a CF titer of 1/240.

Listeriosis is considered responsible for about 1% of neonatal deaths. Early diagnosis and treatment appears essential to reduce the mortality figure as well as to prevent a possible nursery outbreak. Secondary infection of other infants and of nursing personnel in the nursery has been observed by others. Diagnosis of listeriosis during pregnancy is discussed. Treatment in-

stituted at this time seems to protect the child against infection.

**DISCUSSION:** *A. Wallgren:* Can *Listeria* infection in mothers be transmitted to their fetuses during subsequent pregnancies? Or is the risk only present when the infection is newly acquired as in toxoplasmosis? Could repeated abortions as in this case be caused by chronic listeriosis in the mother? — *A. Alvin:* Yes, there are case reports suggesting this. The theory is that the agents colonize in the vaginal mucous membrane. Seeliger has reported 8 patients with habitual abortions in whom serological evidence of *Listeria* infection was found. After antibiotic treatment 6 of the women gave birth to normal, healthy children.

*I. Alm, G. Rodhe and F. Edvall:* Current social and medical problems in mothers' homes

**DISCUSSION:** *A. Wallgren:* It is regrettable that all training schools for unmarried mothers in Stockholm are now transformed in mothers' homes. These training schools gave these young unmarried and inexperienced mothers a proper training for the care of their child and in house-keeping. In the past there was much discussion if mothers should be forced to stay in these institutions during the whole breastfeeding period or if they should have free choice of how long they want to stay in the institutions.

The community now, unfortunately, has taken away a great part of the mothers' responsibility as regards breastfeeding and the general care of the child. I think we pediatricians agree that this trend is not beneficial for the child.

#### Meeting April 8, 1960

*B. Ivarmark and L. Ström:* Cachexia resulting from infundibuloma.

The patient was born at term and developed normally until the age of four months. She then became anorexic and lost weight. At the age of seven months she was admitted

to the Samariten Children's Hospital in Stockholm. On examination she weighed 4500 g, was poorly developed but was otherwise normal. Routine laboratory examinations of blood, urine and feces were also normal. The electroencephalogram was suggestive of a right-sided brain lesion. She



remained in the hospital for 15 months and during this time gained only 2000 g. She died at the age of 22 months without any definite diagnosis having been made. On post-mortem examination a grey, lobulated, sharply demarcated, non-capsulated tumor the size of an orange was found in the region of the thalamus and the hypothalamus. On section, the surface was homogeneous, moist and some areas were gelatinous and vascular. On microscopic examination the tumor was highly vascular with uni- and bipolar cells containing fusi-form nuclei. In the gelatinous areas mucoid degeneration and large round cells with eosinophilic granules resembling histiocytes were found. Some eosinophilic round formations without nuclei were also detected but no deposition of mineral salts had occurred. The structure of this tumor corresponds to that described as an infundibuloma by Globus (*J Neuropath Exp Neurol* 1:59, 1942). The clinical picture is generally that of a diencephalic syndrome with onset in early infancy. The course is often protracted and characterized by euphoria and cachexia. Endocrinological symptoms are rare.

**M. Gullmar and A. Roch-Norlund:** A case of meningococcal meningitis with uncommon complications

A ten-year-old, normally developed boy, who was known to have a systolic murmur possibly due to ventricular septal defect, became ill with symptoms of meningococcal meningitis and was admitted after two days. At admission he had cutaneous bleeding but his general condition was rather good and there were no signs of shock. Treatment was immediately started with large doses of penicillin and sulfa and after three days the temperature was normal and the skin lesions

healed. About 36 hours later fever recurred associated with signs of pleurisy, pericarditis, myocarditis and possible endocarditis, and arthralgia and swelling of the large joints including the elbow. The antistreptolysin titre was 1100 IU/ml; however no hemolytic streptococci were cultured from the throat. In about two weeks the patient improved, was afebrile and the ESR was only slightly elevated. The antimicrobial dosages were reduced but 10 days later the patient again developed high fever, arthralgia and signs of carditis. The doses of penicillin and sulfa were increased without effect, following which cortisone was added and the symptoms quickly subsided. The differential diagnostic possibilities include: 1, septic metastases; 2, toxemia; 3, drug allergy; 4, toxic allergic reaction resembling rheumatic fever.

**DISCUSSION:** *E. Bengtsson:* A case of meningococcal meningitis associated with arthritis and myocarditis was seen this fall at the Hospital for Infectious Diseases. Signs of residual cardiac involvement are still present. In 1953 during the epidemic of paratyphus fever among 800 consecutive cases of paratyphus fever, type Breslau, 10 patients had an associated polyarthritis with high sedimentation rate. None presented an elevated ASLO titer and none had any evidence of cardiac involvement either during the acute episode or at follow-up 4 to 5 years later. There was never any reason to suspect rheumatic fever. It is extremely rare that any organism other than streptococcus is associated with rheumatic fever. The case presented here may be an exception. On the other hand, such marked elevation of the ASLO titer even if not significantly increased makes it difficult to rule out a streptococcal infection.

Meeting May 7, 1960

**O. Celander:** Studies of the peripheral circulation in newborns. Published in *Acta Pædiat*, 49: 488, 1960.

**O. Celander and G. Thunell:** Method for measuring the blood pressure in newborn infants. Published in *Acta Pædiat*, 49: 477, 1960.

**J. Bjure, G. Lidén, T. Reinand and A. Vestby:** Follow-up study of icteric fullterm infants without hemolytic disorders. Will be published in *Acta Pædiat.*

**L. Nilsson:** Fanconi's anemia and various types of this syndrome. Published in *Acta Pædiat.*, 49: 518, 1960.

**H. A. Hansen, R. Jagenburg, B. Johansson and T. Reinand:** Hemoglobinopathia M. Published in *Acta Pædiat.*, 49: 503, 1960.

**H. Forssman and O. Lehmann:** Chromosome studies in mongolism. Published in *Acta Pædiat.*, 49: 536, 1960.

#### Meeting Sept. 26, 1960

**Hypoglycemia in childhood (Symposium at the Children's Hospital, Gothenburg).** Members of the panel: O. Broberger, O. Lehmann

and B. Redin. Moderator: Prof. R. Zetterström. Will be published in *Acta Pædiat.*, 1961.

#### Meeting Sept. 28, 1960

**Garin C. Arneil (Glasgow):** Traumatic osteodysplasia

Ten infants (3 males, 7 females), whose ages ranged from 1-24 months, are described in whom a degree of trauma varying from unrecognised to considerable led to a typical pattern. The condition was variously misdiagnosed as scurvy, neonatal osteitis, hemophilia, cortical hyperostosis, leukemia osteitis and congenital syphilis by senior experienced clinicians. The pattern of disorder is a minor fracture involving the diaphyseal portion of the metaphysis. Extensive subperiosteal hemorrhage occurs and strips the loosed attached periosteum. Squaring and calcifying of the subperiosteal hemorrhage leads to gross deformity of bones which may take years to heal. Multiple deformities are common and may be seriatim rather than concurrent. By a process of exclusion it has been concluded that this pattern may be due either to undue trauma on a normal skeletal system or slight trauma where osseous stability is impaired. The stages of the disease, an abnormal response of normal osseous tissue, are: (1) traumatic damage to the diaphyseal plate, (2) considerable subperiosteal hemorrhage, (3) calcification of hemorrhage and squaring of ends of bone, (4) bizarre pattern of metaphyseal structure and (5) recovery.

**W. F. Sweetnam (Huddersfield):** Congenital fibrosis of the liver as a familial defect

Congenital cystic disease of the liver is well known. A variant of the condition,

congenital fibrosis of the liver, is becoming increasingly recognised. In congenital fibrosis of the liver there is an increase of fibrosis in the portal tracts together with an excess of small bile ducts, the zonal pattern being otherwise normal. It seems likely that the progressive dilatation of the bile ducts and the attenuation of the fibrous tissue finally gives rise to the typical polycystic liver. There may be an associated polycystic disease of the kidneys (Melnick 1958, Parker 1955) and of the pancreas (Ivemark *et al.* 1959). The syndrome may present at birth or may be an incidental finding at autopsy. Sherlock (1960) has records of two families in whom all the siblings are affected. This report describes the condition in all three children of one family. Biopsy in each case showed a typical picture of congenital fibrosis of the liver. The first two cases presented at the ages of two and five with hematemesis. The presence of portal hypertension, esophageal varices and portal-systemic collateral circulations were demonstrated by percutaneous transplenic portal venography. Both children were protected from further hemorrhage by porta-caval side-to-side anastomoses and have remained well for 6½ and 7 years. One of these children has polycystic kidneys. The third child died at the age of six months from pyelonephritis due to polycystic kidneys. It is suggested that congenital fibrosis of the liver is a genetically determined defect and may be dominant.

**Diabetes in children and adolescents (Symposium at the Crown Princess Lovisa's Chil-**

*dren's Hospital, Stockholm*). Members of the panel: Y. Larsson, G. Sterky, K. M. Herrlin, T. Möller, U. Otto and B. Person.

Moderator: Prof. C. Gyllenswärd. The principal part of the symposium will be published in *Acta Pædiat.*

#### Meeting Sept. 29, 1960

##### **W. Gaisford (Manchester): The place of quadruple antigens in immunization**

Multiple antigens are essential in any mass immunization schedule and the advent of poliomyelitis vaccine has enabled the well-accepted triple antigen (D.P.T.) to become quadruple. There is already ample evidence that these four antigens can be satisfactorily combined. There are two main problems; the first is the earliest age at which immunization can be started with the quadruple antigen. In our studies we have shown that, with the triple antigen, good results follow after three doses given at 1, 5 and 9 weeks of age, if the antigen contains aluminium phosphate. Unfortunately, equally good results have not followed poliomyelitis immunization at this period of infancy, partly because of the inhibitory effect of placentally transmitted antibodies and partly because of the poor antigenic property of the existing Type I component of the vaccine. The second problem is therefore the provision of a vaccine with a purified and concentrated Type I component. We have been using such a product recently and the results so far are most promising; a three dose primary course is essential at this age, and,

with added Type I potency, maternally transmitted antibody inhibition can be successfully overcome. The booster response at 1 year in infants receiving three doses of poliomyelitis vaccine at 1, 5 and 9 weeks of age is excellent. There is no reason why quadruple antigen should not be given routinely at 1, 5 and 9 weeks and a booster at 1 year. This would enormously simplify the immunization schedule.

In the *discussion* Professor Gaisford said that pertussis was the most important of the four components in earliest infancy and that unfavourable reactions were minimal at this age. The risk of pertussis in the first 6 months of life was likely to be far greater than the risk of complications following immunization and he felt very strongly that pertussis vaccine should be included in all the schedules of early immunization.

*Ulcerative colitis in children and adolescents (Symposium at the Pediatric Clinic of Karolinska sjukhuset, Stockholm)*. Members of the panel: O. Broberger, L. Billing, R. Lagercrantz, H. Rosengqvist and U. Rudhe. Moderator: Prof. J. Lind. Published in part in *Acta Pædiat.*, 49: 810, 1960.

#### Meeting Sept. 30, 1960

##### **J. Lorber (Sheffield): The development of hydrocephalus in infants born with meningo-myelocele and encephalocele**

Air ventriculography was routinely performed in a consecutive series of 115 infants who were born with meningo-myelocele and encephalocele to determine the true incidence of hydrocephalus and to observe the prognosis of those who had a normal ventricular system in early infancy. The meningo-myelo-

cele had been repaired as a surgical emergency soon after admission, mostly in the first week of life (81 infants or 70%). The ventriculograms were performed within 3 weeks of this operation in 69 infants. The average period between operation and ventriculography was approximately 30 days. Hydrocephalus was found in 94 infants (82%). The incidence of hydrocephalus was highest in infants whose meningo-myelocele involved the lumbar region (79 out of 90

(88%) in infants with paraplegia (72 out of 78 (92%)), and in infants whose head circumference at the time of the ventriculogram was over the 90th percentile (61 of 64 (95%)). The combination of all three of these factors showed that almost all such infants had radiologically demonstrable hydrocephalus in early infancy. The incidence of hydrocephalus in infants whose meningo-myelocele did not involve the lumbar region or had no paraplegia was much less, but even in these, and even if the head circumference was small or normal, the incidence was still high (57% and 65%, respectively). None of the 21 infants who had no hydrocephalus in the neonatal period developed hydrocephalus later. Their mental development was normal, except for the three who had an encephalocele. Repair of the meningo-myelocele did not lead to the development of hydrocephalus in any infant. The hydrocephalus, if present, is part of the neurological syndrome at birth and is not related to operative treatment, nor is it a delayed feature, although clinical signs may not be present for several weeks or months.

**O. H. Wolff (Birmingham): Veno-occlusive disease (seneciosis) and protein-losing enteropathy**

In August 1958 a 2½-year-old boy developed generalised edema, ascites, pleural effusions and hepatomegaly. The serum albumin was 1.7 g/100 ml; the globulin fractions were also depleted with a gamma globulin of 0.3 g/100 ml. The hypoproteinemia was not the result of a deficient intake of protein. Tests of gastro-intestinal function, a barium meal and enema and a jejunal biopsy were normal and malabsorption was thus unlikely. Tests of parenchymatous liver function were also normal and there was no proteinuria. In January 1959 a laparotomy and liver biopsy were performed. These showed yellowish fluid in the peritoneum, the omentum was edematous, the spleen normal and the liver grossly enlarged with a smooth surface. The histology of the liver suggested the diagnosis of venoocclusive disease. The

centrilobular veins were partially occluded by a loose collagenous reticulum in their walls. The surrounding sinusoids were dilated and engorged with blood. It then came to light that the patient was in the habit of eating various plants, his favorite being groundsel (*Senecio vulgaris*). With the object of preventing the development of cirrhosis—a recognised complication of veno-occlusive disease—the patient was given a course of prednisolone. The effect was surprising: within a week of starting prednisolone in a dose of 25 mg daily, the serum albumin had risen from 2.2 to 4 g/100 ml. The globulin fractions took 3 weeks to return to normal. There was a quantitative relationship between the dose of prednisolone and the albumin level, so that when the dose was reduced to 5 mg daily the albumin fell to 3.2 g and when the dose was increased to 10 mg the albumin rose again to 4 g/100 ml. After 3 months prednisolone was discontinued; the edema reaccumulated and steroid therapy had to be restarted. In March 1960 a repeat liver biopsy showed great improvement; the centrilobular veins were normal and there was only slight distension of the sinusoids. While still on prednisolone the patient was given an intravenous dose of I<sup>131</sup> labelled polyvinylpyrrolidone (P.V.P.). At the time of this test the serum albumin was 4 g/100 ml. Only 1% of the dose of P.V.P. appeared in the feces; this is a normal result. Prednisolone was then discontinued and when the albumin level had fallen to 2.5 g/100 ml a second dose of P.V.P. was given and 12.5% of the dose appeared in the faeces. This gross leakage of protein into the alimentary tract was considered to be responsible for the hypoproteinemia. The response of the protein-losing enteropathy to steroids reminds one of the situation in nephrosis where the protein leak through the glomerulus responds similarly. The relationship between the protein-losing enteropathy and the veno-occlusive disease remains obscure. It is conceivable that some of the unexplained cases of protein-losing enteropathy will be shown to be instances of veno-occlusive disease (seneciosis). However,

another and possibly more likely explanation is that this patient suffered from primary protein-losing enteropathy and that the constant drain of protein predisposed his liver to the toxic action of the pyrrolizidine alkaloid in the groundsel.

*Hemolytic anemias in the Pediatric age group (Symposium at the Pediatric Clinic of Akademiska sjukhuset, Uppsala).* Members of the panel: L. Garby, C. Högman, A. Killander, S. Sjölin and C. de Verdier. Moderator: Prof. B. Vahlquist. Will be published in *Acta Pædiat*, 1961.

### Meeting Oct. 14, 1960

**B. Hellström:** Aspects of the diagnosis of rheumatoid arthritis in children (to be published in *Acta Pædiat*)

**H. Herzenberg and V. Eskelund:** The morphological development of pulmonary arteries during the first years of life

In order to show the normal morphological development of intrapulmonary arteries during the first years of life, lung preparations from 66 children ranging from birth to 5 years were examined microscopically. These children had died of disease not primarily affecting the respiratory or circulatory organs. The vessels were classified in four groups according to size and structure. Diameter of the lumen and thickness of the vessel wall were measured and the ratio between these two was calculated. At birth and several days thereafter, the pulmonary arterial bed has a characteristic appearance with narrow luminae and thick vessel walls. The ratio is low. During the first months of life the lumen enlarges and the vessel wall decreases in width, resulting in an increased ratio. This change takes place more slowly during the first weeks of life than later on. This helps to prevent a large left-to-right shunt through an open ductus which might result in left ventricular failure. At heart catheterization and angiocardiography a left-to-right shunt through an open ductus has been shown to exist up to two weeks of age. As soon as the ductus was closed, the widening of the lumen and the thinning of the vessel wall occurs more quickly. After the fourth month of life, this transition occurs more gradually and by the eighth month of life has been largely completed.

By then, the vessels of the lungs resemble those characteristic of the adult, in whom the pulmonary vascular bed is adjusted to a circulation with low resistance.

**P. Zetterqvist:** Familial atrial septal defects

Data on several cases of atrial septal defect of the secundum type in one family is presented. The pedigree suggests a dominant mode of inheritance in four generations with eight certain and five suspected cases. In four cases the diagnosis was confirmed by anatomical inspection of the heart, three at the time of surgical correction. Cytogenetic studies have shown chromosomal aberrations varying in type in different individuals. These findings are similar to those reported in mongolism and suggest cytotypic and probable pathogenetic differences. In a healthy member of the family, an aberration in the form of a balanced translocation has also been found. The pathogenesis of chromosomal aberrations is discussed.

**L. Gottfarb, R. Lagercrantz and A. Lagerdahl:** Sleep disturbances in infancy and early childhood

A child psychologist, a pediatrician and a public health nurse collaborated at the Norrtull Well-Baby Clinic in Stockholm. Every fourth mother to children between the ages of 9 months and 4 years was asked if her child had presented sleep disturbances which had troubled or disturbed the family. Ninety-three mothers were asked and 89 answered. Twenty-two (25%) stated that their children had had insomnia for at least 4 weeks during

the preceding 6 months. Sleep disturbances were specially common below the age of 2 years. Difficulties in falling asleep were more common among boys than girls. The latter awakened more often at night. Most children had had insomnia for more than 7 months. Children with sleep disturbances were compared with those without in the initial sample as well as with a selected material of social twins without sleep disturbances. The comparison was based on the results of interviews with mothers whose relationship and attitude to their children was graded. The results were analyzed statistically primarily with the "sign test". Social status, family situation or lodging was not found to have any significant effect on the incidence of insomnia. A more or less significant relationship was established between insomnia in the child and the following:

feeding difficulties, undue liveliness in the child, anxiety in the mother and inconsistent handling of the child. Several factors such as feeding difficulties, frequent infections, tiredness in the mother and inappropriate attitude toward the child were often present in the same family. Collaboration between child psychologist, pediatrician and public health nurse was found to be of significant value in the handling of these cases.

*R. Lagercrantz, Stockholm*

### Erratum

In order to avoid misinterpretation it should be noted that the word *rubeola* in the report of the Swedish Pediatric Society's proceedings of Feb. 12, 1960 (*Acta Pædiat* 49: 653, 1960) means *German measles (rubella)*.



## ANNOUNCEMENTS

## The Nestlé and the Guigoz Fellowships

These fellowships are granted every year to physicians who wish to perform special research work in the field of nutrition. The fellowships for 1960-61 will be awarded in the spring of 1961 by the International Children's Centre, Paris. The candidates are requested to send the following papers to the Centre: (1) a curriculum vitae, mentioning work on biological and social problems concerning feeding and nutrition of infants and children, (2) a letter of introduction from one of their chiefs, (3) a description of the studies which they wish to pursue with the help of the fellowship. The candidates must have a sufficient command of the French language. Further information can be obtained from the Centre. Address: Château de Longchamp, Bois de Boulogne, Paris XVI.

## International Children's Centre, Paris

The following post-graduate courses and seminars will be organized by the Centre in 1961.

## Courses in France

1. Course on the *Development and Behaviour of the Child* for educational personnel of elementary schools (Paris, January 9th-February 19th).
2. Course on *Child Nephrology* for physicians (Paris, February 20th-March 5th).
3. Course on *Vaccinations* for physicians (Paris, March 6th-26th).
4. Course of *Social Pediatrics* for physicians (Paris, April 10th-July 2nd).
5. Course on the *Prevention and Treatment of Tuberculosis in the Child* for physicians (Paris, September 11th-October 18th).
6. Course on *Mother and Child Welfare* for medico-social personnel (Paris, October 9th-December 17th).

7. Course on the *Organizational Problems of Tuberculosis Control* (in English) for the British Commonwealth physicians in training at the University of Cardiff (Paris, 10 days in November).
8. Course on the *Medico-Social Problems Raised by Chronic Diseases* of the child for physicians (Nancy, March 6th-26th).
9. Course of *Social Psychiatry* for physicians (Lyons, at the end of the year).

## Courses in Africa

1. Course on *Public Health Problems* applying to childhood for physicians (Tananarive, April 10th-May 7th).
2. Course on *Mother and Child Welfare* for welfare personnel (Dakar, 4 weeks).

## Courses in Latin America

1. Course on *Problems of Feeding, Nutrition and Digestive Pathology*, in Buenos Aires (6 weeks in autumn).
2. Participation in a course of *Social Pediatrics* organized with the cooperation of the WHO and the Panamerican Health Bureau for physicians working in the Chilean MCW services.

## The Thirteenth Nordic Congress of Pediatrics

The 13th Nordic Pediatric Congress will be held in Copenhagen June 25-28, 1961. The principal themes to be discussed are: I. The Importance of Prenatal and Natal Factors for Development and for Diseases in View of Prospective and Longitudinal Studies.—II. Epilepsy in Childhood.—III. Steroid Treatment of Acute Life-Endangering Infections. IV. The Prognosis of Asthma and Asthmatic Bronchitis in Childhood.

There will be a scientific exposition in connection with the Congress.

The address of the General Secretary: Rigshospitalets Børneafdeling, Blegdamsvej 5, Copenhagen Ø. Denmark.



BOOK REVIEWS

**Maurice Lamy and Pierre Maroteaux: Les Chondrodystrophies Genotypiques.**

L'Expansion Scientifique Française, Paris, 1960. 120 pages, 100 figures. Price 1 NF.

This is a well-illustrated, informative book giving a detailed description of the various types of systemic congenital disorders of the skeleton. The classification of these osteochondrodystrophies is based upon the clinical signs and symptoms, the radiological findings and the mode of transmission. The differential diagnostic discussions increase the value and usefulness of the book.

**Bensaude, A. and Chigot, P. L.: Précis de proctologie infantile, L'Expansion Éditeur, Paris 1960. Price 11.50 NF.**

Ano-rectal lesions are of relatively infrequent occurrence in childhood. Pediatric textbooks give little information in this field. This monograph presents concisely in 120 pages, the essential facts on childhood proctology. The instrumental and technical requisites for adequate proctological examination are described. Etiology, pathology, clinical picture and treatment of the main proctologic disorders in children are discussed.

*Th. Ehrenpreis, Stockholm*

**R. S. Illingworth: The Development of the Infant and Young Child, Normal and Abnormal.**

Livingstone Ltd., Edinburgh and London, 1960. 318 pages, 95 figures, 4 tables. Price 27s. 6d.

The author says that a book on child mental development should be written by a paediatrician, not by psychologists. The

reviewer feels that he is right. Too many books on this subject have been published by people who have only little knowledge of the child as a whole, in health and disease. The book solely treats mental development of the pre-school child, the knowledge of which Illingworth feels is as fundamental to the paediatrician as is anatomy to the surgeon. He tells the physician in simple language what can be learnt about a child's development with a minimum of equipment and without special training. He discusses the significance of prenatal and perinatal effects, physical defects and environmental factors upon the mental developmental rate of children. He emphasizes the wide variation in development as a whole and defines the limits of our predictive powers when using the tests presently available. These tests are described in detail and their interpretation discussed. An especially valuable and interesting chapter is that on "Mistakes and Difficulties in Developmental Diagnosis". Each chapter of the book is followed by a bibliography. This book fits very well in the row of previous excellent volumes on child care and welfare and child development written by Professor Illingworth.

**Sir James Spence: The Purpose and Practice of Medicine.**

Oxford University Press, London. 1960. 308 pages. Price 42 s. net.

This book contains 20 selections from Sir James Spence's bibliography of 53 publications. The articles are well chosen. It is with profit and great enjoyment that one reads these papers, which reflects so well the charm of their author, the personal, un-doctrinal way in which he tried to solve problems, the vast variety of his interests in the field of medicine, his broad-minded,

humanitarian aspects on the medical profession and his great common sense. The reviewer wants to call especial attention to those readers who do not know the important achievements of Sir James and for whom he is only one of the famous names in the history of pediatrics in Europe, to study carefully the following articles: Nature of Disease, Children and Families, Care of Children in Hospitals and the Need for Understanding the Individual. It is most fortunate that the Editors have accomplished this collection of Professor Spence's papers, thus facilitating references, even to articles that originally were published in journals that are not easily accessible.

One reads with great pleasure the vivid picture of Sir James by Sir John Charles in his introductory 25 pages biography, in which are included some written souvenirs of Spence's friends. His successor, Professor Donald Court, call him "artist and scientist, romantic and realist, conservative and rebel, always a leader". He was without doubt one of the great physicians of this century. He has contributed much to the advancement of our knowledge in pediatrics, especially its social implications. He was a rarely talented man, who used his own way in looking at problems and in trying to solve them and in developing the science and art of being a physician for children. This book should be included in every private pediatric and official library.

**J. F. de Wijn and J. H. de Haas:** Growth Diagrams from 1-25 Years in the Netherlands.

Nederlands Instituut voor Praeventieve Geneeskunde, Leiden. 1960. 30 pages. Price fl. 2.50.

This comprehensive report is written in Dutch but an English summary and English translation of the text of the 11 growth curves facilitate the reading. The study is a cross-sectional one of the height, weight

and secondary sex characteristics of 3798 boys and 8112 girls of average social class. The averages are at a higher level than in former times and it is believed that in the near future the children will be taller and heavier than the children of today. It is planned to complete this investigation with a longitudinal study.

**Moshe Prywes (ed.):** Medical and Biological Research in Israel.

Rubin Mass, Jerusalem, Israel. 1960. 562 pages, numerous illustrations. Price \$8.

This nice volume is divided into eleven sections of which the sixth (Experimental and Clinical Research in Medical Disciplines) contains two pages related to research in pediatrics. Report is given of studies in malnutrition and diarrhea (electrophoretic patterns of serum proteins, serum levels of vitamin A, intestinal flora and plasma prothrombin level), of diseases of the heart (incidence of congenital and rheumatic heart disease, an outbreak of Fiedler's interstitial myocarditis, Cocksackie-virus as cause of heart disease), and of the newborn infant (early jaundice due to ABO incompatibility, evaluation of the state of maturation of the nervous system by means of studies of the reflexes). The reader is impressed by the great activity and high standard of research in Israel.

**T. Yoshida and J. Huizuiga:** Abstracts of Japanese Medicine.

Excerpta Medica Foundation, Amsterdam. 1960. Annual subscription rate \$30.

A new journal reviewing about 500 current Japanese periodicals has just been published. The pediatric part of the journal, which in this first issue contains eight of the total 187 pages, is divided into 28 different sections, covering all items within the pediatric field of medicine. *Acta Paediatrica* wants to draw the attention of its subscribers to this new service of Excerpta Medica.

Institute for Medical Genetics, University of Uppsala, and Pediatric Clinic, Karolinska sjukhuset, Stockholm, Sweden

## Association Between Congenital Heart Malformation and Chromosomal Variations<sup>1</sup>

by J. A. BÖÖK, BERTA SANTESSON and PER ZETTERQVIST

Knowledge of chromosomal variations in man as a cause of abnormal development and disease has increased rapidly during the past two years. At the present time about twenty-five conditions associating numerical or structural chromosomal variations and pathological conditions have been reported; about half of them concern the sex chromosomes and the rest the autosomes. Even if some of the observations are still represented by single individuals only, there is no doubt that clinical cytogenetics, in a very short time, has been established as a new and important branch of human pathology and clinical medicine.

Practically all individuals on record who lacked one chromosome,<sup>2</sup> e.g. XO in Turner's syndrome (8) or who had an additional chromosome such as trisomic 21 in mongolism; trisomic [16-18] in Harnden-Patau's syndrome (7, 11) and trisomic [13-15] in Patau's syndrome (11),

have shown a multiplicity of developmental and morphological changes.

Exceptions from these findings of multiple changes are two individuals with congenital heart defect (1, 5) who will be dealt with in some detail in this paper and an apparently healthy father of a child with mongolism reported from this laboratory by Fraccaro *et al.* (9). This man, who had 47 chromosomes, might be trisomic [19-20], but unfortunately no clinical examination was made.

Malformations of the heart have been a frequent feature of the "chromosomal" syndromes mentioned above. This and the fact that one of us (Zetterqvist) has been particularly interested in pediatric cardiology was the reason why we started to collect information on the karyotypes of individuals with congenital heart malformations.

One of us (Zetterqvist (13)) has reported an exceptional pedigree with eight certain and five probable cases of atrial septal defect of the secundum type. The distribution of affected and normal individuals in this pedigree appears to agree with a simple dominant mode of inheritance. We have now been able to study the karyotypes of one family belonging to this pedigree.

<sup>1</sup> Aided by grants from the Swedish Atomic Research Council and the International Atomic Energy Agency.

<sup>2</sup> The Denver nomenclature of human mitotic chromosomes (6) will be used consistently in this paper (cf. Fig. 1). The expression trisomic is used for brevity although it is noted that this diagnosis is based on the morphological similarity of mitotic chromosomes only.

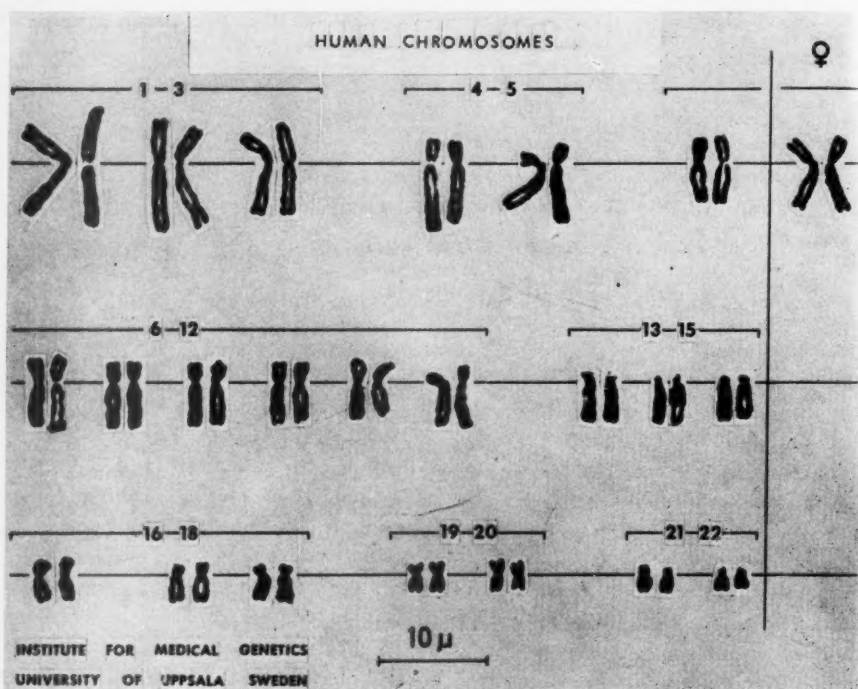


Fig. 1. The normal female chromosome set arranged in accordance with the Denver agreement.

#### *Description of the family*

The mother, E. G., was aged 49 (No. III:6 in Zetterqvist's (13) original pedigree). She has had dyspnea on moderate exercise since childhood. Her condition deteriorated in 1957 following an attack of influenza. At the special examination in October 1959, which included catheterization and angiocardio-graphy, a large auricular septal defect of the secundum type was found. This defect was associated with mitral insufficiency due to relative dilatation of the mitral ring without cusp malformations. The relative heart volume was 900 ml per sq.m body surface area. The blood flow through the pulmonary and systemic circuits was 34 and 4 l per minute, respectively. Both malformations were corrected at an

operation in November 1959 at the Chest Clinic in Stockholm (Professor C. Crafoord, M. D.). The postoperative course was uneventful and the patient is now (June 1960) in good health.

#### *Cytological observations on E. G.*

*Method.* Chromosome studies were made according to the technique developed in this laboratory (3). The cytological observations on the patient E. G. are based on four separate cell cultures from a bone marrow biopsy taken Oct. 21, 1959, and four separate cultures from a skin biopsy taken January 15, 1960. At the preparation of the skin biopsy two cultures only were started from trypsinized material. The other two

TABLE

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TABLE 1. *Summary of chromosome findings in a family in which mother and son had an atrial septal defect of the secundum type.*

	Individual and origin of cultured cells	Chromosome counts				Total number of analysed cells	Chromosome complement
		45	46	46±1	47		
E.G.	Mother, bone marrow	4	4	0	25	33	Trisomic [19-20]; XX
	Mother, skin	2	6	0	17	25	Trisomic [19-20]; XX
C.G.G.	Father, bone marrow	0	8	0	0	8	Normal; XY
C.M.G.	Son, bone marrow	1	19	1	0	21	Trisomic [19-20]; Monosomic 22; XY
	Son, skin	0	10	1	0	11	Trisomic [19-20]; Monosomic 22; XY
I.G.	Daughter, bone marrow	0	9	0	0	9	Normal; XX

were prepared by mincing the material directly in the medium with a pair of fine ophthalmological scissors. This process is apparently less damaging to the cells. By omitting the initial trypsinization for skin, muscle and fascia lata biopsies the time lag between the preparation of the culture and the first signs of growth has been shortened to an average of 14 days for adult material.

The bone marrow cultures have been observed over a period of 2 months. Microscopic slides were prepared from primary as well as trypsinized and transferred cultures. The skin cultures have been studied in the same way for a period of about three months. As the results from the examination of the different cultures and subcultures were all consistent they will not be itemized. We have carefully selected and examined 58 apparently undamaged cells in mitotic metaphase derived from the skin and bone marrow of E. G.

The results of the chromosome counts have been summarized in Table 1. The majority of the cells contained 47 chromosomes but by our standards there were too many quite clear metaphases with 46 to be accounted for by technical biases.

Five 47 chromosome cells derived from the bone marrow and three derived from the skin have been analysed in detail by

the aid of enlarged photomicrographs. In all these cells we found five short chromosomes with nearly median centromeres. Thus, on morphological grounds the additional 47th chromosome was classified as belonging to group [19-20]. In Fig. 2 which exemplifies the karyotype this additional chromosome has been placed at position 19. Tentatively these cells were diagnosed as trisomic [19-20].

Two 46 chromosome cells were likewise analysed in all details. Matching of the chromosomes showed a normal complement with two X-chromosomes. Also in the remaining 46 chromosome cells only four short chromosomes with nearly median centromere could be recognized.

The 45 chromosome cells (cf. Table 1) displayed no consistent chromosome pattern and are most reasonably explained as due to the accidental loss of one chromosome when the squash preparations were made.

The husband, C. G. G., born in 1910, is a normal healthy male without signs or symptoms of cardiac disease. There is no consanguinity between him and his wife.

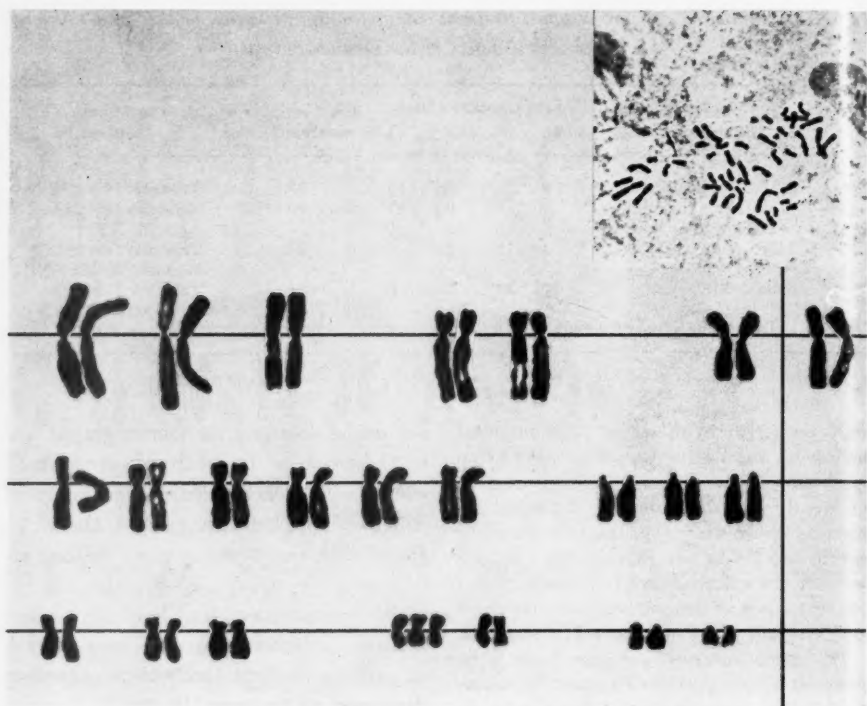


Fig. 2. The chromosome complement of the mother with congenital heart defect, apparently trisomic [19-20].

Eight carefully analysed cells in mitotic metaphase, derived from three separate bone marrow cultures, showed a normal male complement with 46 chromosomes, as exemplified in Fig. 3. This couple has two children, a boy born in 1948 and a daughter born in 1950. There are no further issues and no abortions.

The boy, C. M. G., on routine examination was found to have signs of heart disease, although he had no subjective complaints. At catheterization and angiocardigraphy an atrial septal defect of the secundum type was disclosed (i.e. the same defect as in the mother). An operation was not considered

necessary as the pulmonary/systemic flow ratio was shown to be 1.5/1 only.

#### *Cytological observations on C. M. G.*

The observations are based on three separate cell cultures from a bone marrow biopsy and two separate cultures from a skin biopsy, both taken on January 15, 1960. Both sets of cultures have been observed over a period of about three months. The results from the primary as well as the transferred cultures were all consistent. Thirty-two cells in mitotic metaphase have been analysed. The results



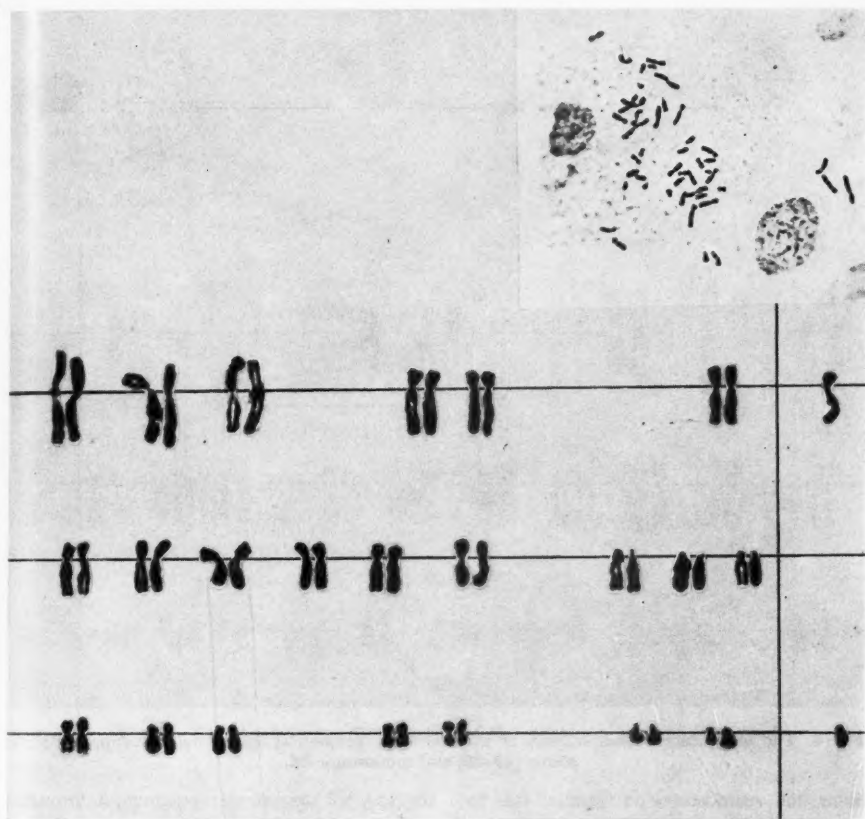


Fig. 3. The chromosome complement of the father, apparently normal.

of the chromosome counts have been summarized in Table 1. Practically all cells in which exact counts could be made (29 out of 30 cells) contained 46 chromosomes. In 15 of these cells with especially clear metaphases, four small acrocentric chromosomes only were found against an expected five in a normal male. Furthermore there were five short chromosomes with median or nearly median centromere (group [19-20]) against an expected four.

Three cells derived from the bone

marrow and four from the skin have been analysed further by the aid of enlarged photomicrographs. All these cells contained a fifth short chromosome with median or nearly median centromere, i.e. apparently the same constellation as in the mother. One small acrocentric chromosome was lacking. As in several cells two small acrocentric chromosomes with satellites (no. 21) and the Y-chromosome could be recognized, it is possible that one chromosome number 22 was lacking. As ex-



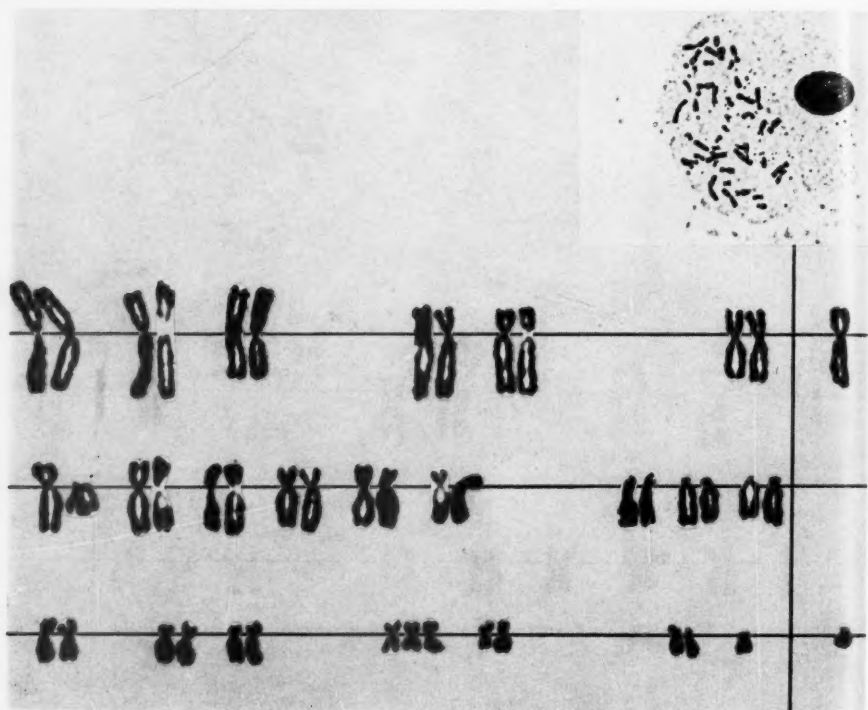


Fig. 4. The chromosome complement of the son with congenital heart defect, apparently trisomic [19-20] and monosomic 22.

emplified in Fig. 4, the karyotype of this individual could be interpreted as trisomic [19-20] and monosomic 22.

The daughter, I. G., was healthy and showed no signs or symptoms of cardiac disease on physical examination, including electrocardiography. A detailed analysis of the chromosome complement in cells from three separate cultures of a bone marrow biopsy showed morphologically normal conditions, as exemplified in Fig. 5.

#### *The diagnosis of individual karyotypes*

It is known that cells cultivated *in vitro* over long periods may change their karyotype. This is presumably due to a number

of different factors operating in a highly artificial environment. However, a considerable amount of the experience with human cell cultures comes from work with malignant cells which originally may consist of a mixture of different karyotypes. The work in this laboratory has been limited to the study of individual karyotypes with the exclusion of all kinds of malignant cells. In laboratories where malignant cell strains are maintained the possibility of infection from such cultures must be considered.

We have found, as have others (1), that open system-fluid medium cultures

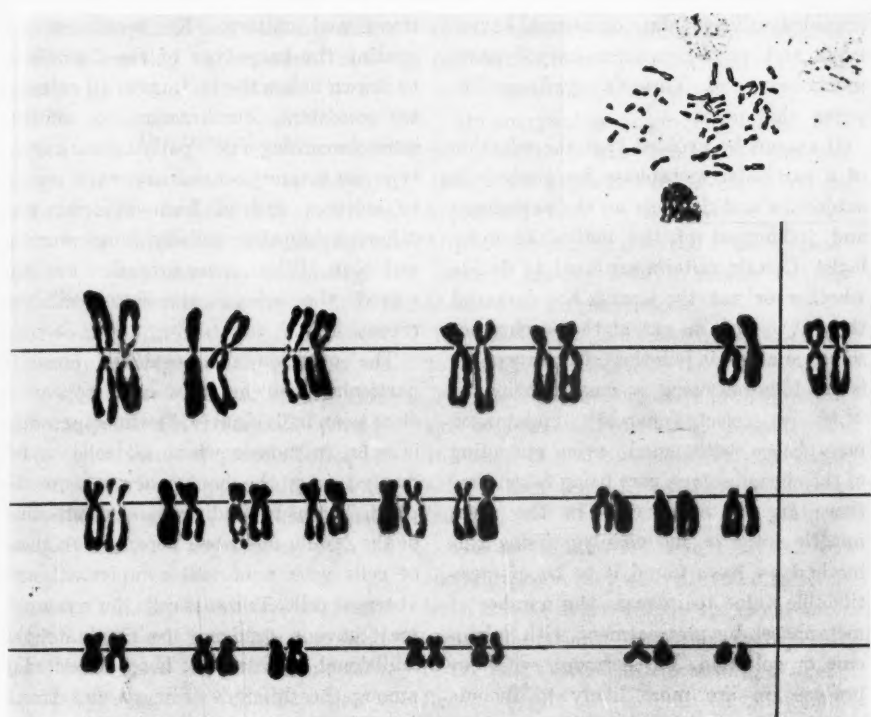


Fig. 5. The chromosome complement of the daughter, apparently normal.

favour karyotype constancy for periods which are long enough to secure a sufficient margin of safety for karyotyping. This has been established in our laboratory during the work with more than 400 cultures from biopsies of about 120 individuals and over 100 cultures derived from human fetuses. In several instances the cultures have been checked for the constancy of the karyotype for periods up to six months. In general the number of polyploid cells with multiples of the diploid number tends to increase after several transfers. Usually this will not interfere with a correct interpretation but may even be a help to establish the identity

of additional chromosomes. Occasional aberrations have been observed, e.g. chromatide breaks, somatical pairing and anaphase disturbances. The incidence of such abnormalities has been estimated not to exceed one per cent of the mitoses and the disturbances did not increase significantly during the time the cultures have been observed. Nevertheless, it is recommended that, whenever possible, karyotyping be made on early cultures and preferably on the primary ones. Minor modifications of the culture medium or technique appear unimportant in this context. A number of independent laboratories, using different techniques, has now

provided sufficient data on normal karyotypes and on the common karyotype in mongolism and Turner's syndrome to prove this point.

It cannot be avoided that the selection of a particular metaphase for analysis is subjective and depends on the experience and judgement of the individual cytologist. Certain criteria are used to decide whether or not the squash has damaged the cell to such an extent that a chromosomal analysis is pointless. Our procedure is as follows: using a magnification of  $\times 160$  we select apparently undamaged metaphases with good, even spreading of the chromosomes, care being taken that there are no other cells in the same mitotic stage in the vicinity. Using this method we have found it to be of questionable value to increase the number of metaphases by pretreatment with colchicine or colcemid. Furthermore, with our process we are more likely to include occasional metaphases in which one chromosome has been lost than those having received an additional chromosome dislocated from a nearby metaphase.

With the cell culture technique it is not possible to prove directly that the karyotype determined for the cells *in vitro* is exactly the same as in the donor's body. The findings must be corroborated by circumstantial evidence. The general process in this laboratory is to start 3-5 independent cultures from each biopsy and determine the karyotype on the primary as well as the transferred cultures over a period of 2-3 months. While it is possible that new karyotypes may arise, e.g. through mitotic non-disjunction, it is extremely unlikely that the same mutation will occur in all the primary and

transferred cultures. No conclusion regarding the karyotype of the donor will be drawn unless the findings in all cultures are consistent. Furthermore, for conclusions concerning a new pathological karyotype we require consistency with regard to cultures derived from at least two different biopsies, usually bone marrow and skin. When inconsistencies are observed the whole procedure will be repeated.

The question of mosaicism presents particular problems. Few cases appear as clear as an individual (4, 5) who apparently is a  $3n/2n$  mosaic where all cells so far derived from the bone marrow were diploid. The usual finding, as in the mother of the family described here, is a mixture of cells with a normal complement and aberrant cells. Trisomic cells, for example, may have a tendency to eliminate the additional chromosome. If so, consistency among the different primary and transferred cultures will not prove the point. There is no way of knowing whether this alleged elimination, if at all occurring *in vivo*, takes place more rapidly *in vitro*. It seems doubtful if karyotyping based on the direct incubation of bone marrow cells will help much. In so far as mitotic activity is concerned the bone marrow is most similar to our *in vitro* cell cultures. Consequently, chromosomal aberrations which tend to interfere with the mitotic mechanism could be more heavily selected against here than in other tissues. It should also be kept in mind that a large number of drugs at therapeutic concentrations and industrial or professional chemicals are known to affect the bone marrow in particular. These effects may have a different selective value on dif-

ferent karyotypes as well as being instrumental in creating chromosomal abnormalities.

### Discussion

From the data referred to above it seems reasonable to infer that the karyotypes of the cultivated cells correspond to those of the donors. By morphological criteria the 47th chromosome of E. G. is similar to the additional short chromosome with nearly median centromere in her son.

The lack of one small acrocentric chromosome in the son is remarkable, particularly in the absence of gross effects. The very consistent karyotype findings in the cells derived from this individual, make it unlikely that we are dealing with an artefact.

The son, C. M. G., would have originated through an egg cell containing an additional chromosome [19-20]. About 50% of the mother's egg cells would be expected to have this constitution. The same egg cell may, due to non-disjunction, have lost chromosome number 22. Possibly the extra 47th chromosome might be a disturbing factor at meiosis. Alternatively, the 24 chromosome egg cell might have been fertilized by a sperm with 22 chromosomes only and lacking number 22 due to non-disjunction in the father.

The origin of the abnormal karyotype of the son could also be explained by a mechanism of translocation involving chromosome number 22. If so, the chromosome interpreted as an extra [19-20] in Fig. 4 might be a 22/[19-20] translocation. Such a translocation could have resulted in a new chromosome indistinguishable from the other members of group [19-20]. Then this alternative implies a partial deficiency

of one chromosome number 22 and a partial triplication of the gene material contained in the chromosome of group [19-20].

On morphological grounds, alone, it is not possible to give priority to either of these two explanations. Both would be consistent with an association between triplication of gene material of the chromosomes of group [19-20] in mother and son if not with simple trisomy.

Several other alternative explanations involving translocations or combinations between translocations and non-disjunction are, of course, theoretically possible. On the basis of now available evidence those offered here seem to us the most likely ones.

This communication should not convey the impression that we consider congenital heart defects in general are due to chromosomal aberrations. Nevertheless, in view of the fact that such defects form a significant feature of several chromosomal syndromes, among which mongolism is the most common, this etiological mechanism appears important in cardiac pathology.

We have been looking for chromosomal aberrations in a few other patients with congenital heart defects but with negative results. Two of them have now been karyotyped and merit mention briefly.

File no. 01-45-60, L. M. A., female, born Feb. 28, 1960, died March 27, 1960. Maternal age 27, paternal age 29. She had one normal brother born in 1954 and one normal sister born in 1957. There were no abortions. The pregnancy was uneventful and the birth weight 3840 gram. She was a blue baby with permanent heart incompensation. The post mortem examination showed deformities of the tricuspid and mitral valves, pronounced hypertrophy and dilatation of the

right ventricle, dilatation of the right auricle and a patent ductus arteriosus. A detailed analysis of the chromosome complement in four separate cell cultures derived from a skin biopsy taken March 12, 1960 showed morphologically normal conditions, i.e. 46 chromosomes including two X-chromosomes.

File no. 01-51-60, S. G. L., male, born Feb. 16, 1951. Maternal age 22, paternal age 24. He had scarlet fever in 1955. At this time a heart murmur was detected. An atrial septal defect of the secundum type was confirmed by electrocardiography, catheterization and angiocardiology. In addition he had multiple minor skeletal deformities of the shoulder girdle and hands. As the blood flow of the pulmonary circuit was more than twice that of the systemic circuit an operation was considered indicated and also performed in 1959. The karyotyping was based on three separate primary cell cultures derived from a bone marrow biopsy taken March 23, 1960. He was found to have an apparently normal male complement.

This patient is particularly interesting because his mother had a similar defect. She was M. H., born in 1929, and had deformities of the upper extremities similar to those in her son, only more marked, and with total absence of her right thumb. On the left hand the thumb was substituted by a small radial finger and the thenar part of the hand was malformed. The electrocardiogram showed marked arrhythmia of an unusual type with extreme sinus bradycardia, frequently interrupted by sino-auricular block and an irregular nodal tachycardia. The ventricular complexes, however, were of the type usually seen in atrial septal defect of the secundum type. X-ray findings practically confirmed the existence of a left-to-right shunt at the atrial level. So far she has not been available for karyotyping.

The etiology of congenital heart defects is apparently manifold and complex (2). Abnormal chromosome complements may be instrumental in some instances, while

gene mutations or external factors operate in others. The findings reported in this paper indicate the necessity of paying further attention to chromosomal variations and the mechanisms lying behind these. It is also obvious to us that further confirmatory work is needed. It is hoped to subject further members of the pedigree to cytogenetic studies later.

As the incidence of meiotic disturbances resulting in numerical and/or structural variations are known to increase with increasing doses of ionizing radiation (10), the question of further human defects due to chromosomal aberrations and at the same time compatible with appreciable fertility merits a particular interest. So far the genetical effects of ionizing radiation on human populations have been thought of almost exclusively in terms of gene mutations. With the present knowledge of at least twenty-five different chromosomal variations apparently causing defects in a substantial number of individuals, the association between these phenomena and different levels of ionizing radiation must be given serious consideration.

### Summary

A family is described in which mother and son both displayed auricular septal defects of the secundum type while father and daughter had no cardiovascular disorders. The karyotypes of these individuals were analysed by the cell culture method using biopsies from bone marrow and skin. The majority of the cells from the mother had 47 chromosomes and she was apparently trisomic [19-20]. A similar additional chromosome of group [19-20] was found in the cells derived from the son.

However, he lacked one of the smallest acrocentric chromosomes. His chromosome number was 46. The father and daughter both had normal karyotypes. The significance of numerical and/or structural chro-

mosome variation in the etiology of congenital heart malformation is discussed with regard to recent developments in clinical cytogenetics.

### References

1. BÖÖK, J. A.: Mongolism and other conditions associated with chromosomal variations. *Acta Soc Med Upsal*, 65: 1-2, 1960.
2. — Heredity and heart disease. *Amer J Publ Health*, Part II, 50: 1-4, 1960.
3. BÖÖK, J. A., FRACCARO, M. and LINDSTEN, J.: Cytogenital observations in mongolism. *Acta Paediatr (Ups)*, 48: 453-468, 1959.
4. BÖÖK, J. A. and SANTESSON, B.: Malformation syndrome in man associated with triploidy (69 chromosomes). *Lancet*, I: 858-859, 1960.
5. — Further studies in clinical cytogenetics. *Acta Univ Lund, Ser 2*, 56: 19-20, 1960.
6. BÖÖK, J. A. et al.: A proposed standard system of nomenclature of human mitotic chromosomes. *Lancet*, I: 1063-1065, 1960.
7. EDWARDS, J. H., HARDEN, D. G., CAMERON, A. M., CROSSE, M. V. and WOLFF, O. H.: A new trisomic syndrome. *Lancet*, I: 787-790, 1960.
8. FORD, C. E., JONES, K. W., POLANI, P. E., ALMEIDA, J. C. DE and BRIGGS, J. H.: A sex-chromosome anomaly in a case of gonadal dysgenesis (Turner's syndrome). *Lancet*, I: 711, 1959.
9. FRACCARO, M., KAIJSER, K. and LINDSTEN, J.: Chromosomal abnormalities in father and mongol child. *Lancet*, I: 724-727, 1960.
10. KAUFMANN, B. P.: Chromosome aberrations induced in animal cells by ionizing radiations. In *Radiation Biology*, ed. A. HOLLAENDER. McGraw-Hill, New York, Toronto, London. Vol. I: 627-711, 1954.
11. PATAU, K.: The identification of individual chromosomes, especially in man. *Amer J Hum Genet*, 12: 250-277: 1960.
12. TJIO, J. H. and PUCK, T.: Genetics of somatic mammalian cells. II. Chromosomal constitutions of cells in tissue culture. *J Exp Med*, 108: 259-268, 1958.
13. ZETTERQVIST, P.: Multiple occurrence of atrial septal defect in a family. *Acta Paediatr (Upps)* 49: 741, 1960.

Received September 12, 1960

Accepted September 26, 1960

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## The Influence of Birth on the Weights of the Left and Right Cardiac Ventricles in Prematurely Born Infants

by AVINASH MITHAL and JOHN L. EMERY

While there have been great advances in the clinical study of heart function in the newborn, by Barclay, Franklin & Prichard (3), and more recently by Lind and his associates (13, 14), anatomical data on the heart structure at this age are scanty and we have been able to find none accurately analysed from the point of view of the relationship of birth, crown-rump length and gestational age.

For over 50 years it has been widely stated that there is atrophy of the right ventricle after birth (16). Reviewing older literature, Vierordt (18) quotes authorities showing virtually the same sizes for the tricuspid and mitral valves at one year of age, but at birth the tricuspid valve in one series is reported as being larger than the mitral valve and in another series as being smaller than the mitral valve. One of the most detailed recent studies of the newborn heart was carried out by Keen (10). He found that the mean weight of the free wall of the right heart was less in his children dying between the age of birth and one year, than in stillborns, although his weight ranges show a complete overlap. He concluded from these absolute weights and their relation to the left ventricle weights that there is an atrophy of about one-fifth in the right ventricle commencing rapidly after birth. It is a wellknown clinical feature that many children dying during the first few months after birth

weigh less and are smaller than many normal full-term infants and mature stillborns. Keen's material came from infants referred to the police mortuary in Cape Town and he states that malnutrition present in his cases made it useless for him to use the weights of the infants in his study. He also remarks that "classification of the newly born infants into premature and full term infants was naturally easy" but he does not state his criteria for such a statement. He discusses the earlier literature on the left and right heart weights with excellent thoroughness but we believe that his rejection of the method of dividing the heart used by Lewis (12) and Hermann & Wilson (8), which is essentially the method we have used, and adopting that of Fulton, Hutchinson & Morgan Jones (7) is unfortunate when applied to hearts undergoing fairly rapid change in shape. We also feel that Keen was unfortunate in having to use hearts for which accurate obstetric histories were not available.

Hort (9) also believes that there is a physiological atrophy of the right ventricle of the premature by the end of the first week. He found this change in a study of both cat and human hearts.

Panisello, Castellanos, Junco & Valladares (17) examined the hearts from 25 premature and 8 full term infants using the technique of Hermann & Wilson (8). They classified their hearts on a weight basis and showed a progressive increase in the weight of both ventricles although the ratio changed.



The relationship between the muscle mass and the output of the heart in the foetus is not certain. Dawes, Mott, Widdicombe & Wyatt (5) suggested that in lambs the left ventricular output exceeded the right ventricular output throughout foetal life and Keen (11) suggested that the same occurs even in the full-term infant heart which shows anatomical right-sided preponderance. The mean muscle fibre diameter of the right heart is greater than the left heart in the foetus (2). Boellaard (4) followed Ashley's work of measuring thickness of the muscle fibres. He showed that there was possibly some slight thinning in the total thickness of the right ventricular wall but any possible thinning of the diameter of the fibres did not account for the apparent "atrophy" and he postulated that the changes after birth were probably chiefly those of dilatation of the cavity. His material and method of assessment are subject to many of the same criticisms as that of Keen's material.

There is very great variation in the relative left and right heart weights at birth and so many factors are involved in the study of such hearts that confusion can only be prevented by separating the various criteria. Emery & MacDonald (6) have shown that analysing the same series of children's hearts against crown-rump length, gestation age and weight of the child, produces a different growth pattern. By means of careful dissection of the left and right ventricles in a series of stillborns, they were able to establish what they felt were the normal weight increments of the cardiac ventricles against gestation age, crown-rump length and body weight for the stillborn child during the latter half of gestation. In dealing with postnatal changes it is obvious that body weight can hardly be used as a criteria for analysis due to the great variability in postnatal change in weight,

and even such variables at birth as inhalation and the passage, or not, of much meconium.

In the present study an attempt has been made to compare the actual weights of the cardiac ventricles in children that had lived for more than 5 days after birth with the weights of stillborn children of the same crown-rump length. Any difference in these two findings would suggest either an increase or decrease in the immediate postnatal rate of growth of the heart muscles.

### Material and Methods

A careful selection was made of the post-mortem reports to find children who had been born before 38 weeks gestation and who had lived for 5 days or more, and in whom there was no gross congenital deformity and no evidence that the child had died directly of a circulatory disease. Eighteen such cases were found after surveying approximately 1500 necropsies during which time all children's hearts had been saved. Eight cases had a crown-rump length of between 25 and 29.5 cm and 10 between 30 and 34.5 cm.

In this study we deliberately excluded children dying between birth and five days for two main reasons. One is that during this period the causes of death are too difficult to be stated with any certainty and the other is that it is the effect of postnatal life that is required, and it would not be certain whether any changes could take place of any significance within two or three days of birth.

These hearts were dissected and the ventricles weighed using the same method as that previously described for stillbirths (6), which method is similar to that used by Hermann & Wilson (8) and Panisello, Castellanos, Junco & Valladares (17). The data from these cases were compared with those of the apparently normal stillbirth series.

Comparison was made between the groups of equivalent crown rump length and *t* tests were applied according to the formula: Difference between means/Standard error of difference between means. The values thus obtained for each of the groups were then related to the values obtained from *n* number of cases located in *t* tables at 0.05 levels. The *n* numbers used being ( $n_1-1$ ) and ( $n_2-1$ ) for each comparable group.

### Results

The individual ventricle weights related both to crown-rump length of the child and to the mean line of the values obtained from stillborn children are shown in Fig. 1. It is obvious that the scatter values in the right and left ventricle charts is different. In the case of the right ventricle the values appear to be evenly distributed around the mean line whereas in the left ventricle the majority are above the line. The statistical analyses of the means of these groups are presented in Tables 1 and 2. These show that there is no significant difference between the right ventricular weights of the stillborns and children who lived after birth, of comparable crown-rump length. On the other hand, there is a significant difference (at the 95% and even at the 99% level) between the left ventricular weights of the "living" and stillborns. This finding would suggest that within five days after

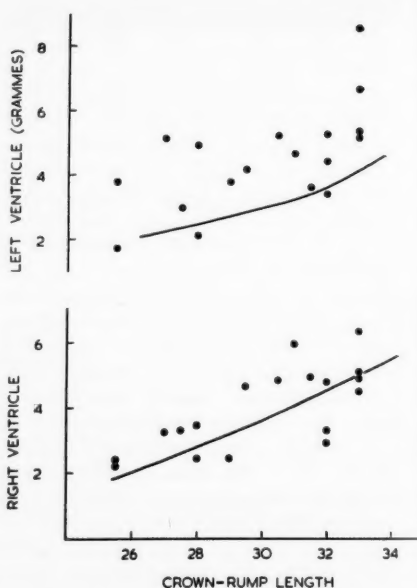


Fig. 1. Showing the weights of the left and right ventricles (shown as spots) against the mean line of a series of stillborns of the same crown-rump length.

birth there has been an appreciable increase in the weight of the left ventricle over that which would have occurred had the child not been born i.e. significant postnatal hypertrophy.

With regard to the right ventricle there is no such deviation from the apparent intrauterine growth pattern. Had there been any postnatal actual atrophy or

TABLE 1. Showing the numbers of cases studied and the mean values obtained together with standard deviation in two crown-rump length groups.

Crown-rump length (cm)	Stillbirths (J.L.E. & M.S.M.)						Livebirths			
	No. of cases ( $n_1$ )	L.V. wt. (g)		R.V. wt. (g)		No. of cases ( $n_2$ )	L.V. wt. (g)		R.V. wt. (g)	
		Mean	S.D.	Mean	S.D.		Mean	S.D.	Mean	S.D.
25-29.5	20	2.4	0.68	2.6	0.58	8	3.60	1.16	2.99	0.77
30-34.5	45	3.7	1.25	4.4	2.04	10	5.37	1.42	4.75	1.7

TABLE 2. Showing the *t* values obtained against *t* values from tables at 1 in 20 probability.

Crown-rump length (cm)	Left ventricle		Right ventricle	
	<i>t</i> value obtained	<i>t</i> from tables <sup>a</sup>	<i>t</i> value obtained	<i>t</i> from tables <sup>a</sup>
25-29.5	2.72	2.056	1.44	2.056
30-34.5	3.42	2.01	0.729	2.01

<sup>a</sup> At 0.05 level.

hypoplasia of this ventricle during this period as postulated by Hort (9) and Keen (10) we would have expected to see lower mean weights in the postnatal ventricles. It may be argued that the postulated atrophy had not had time to commence in our material but the positive find of hypertrophy of the left ventricle in the same hearts does suggest that a change in the opposite direction, if present, would have been revealed.

We feel justified in suggesting that there is no evidence for postnatal atrophy of the right ventricle.

### Summary

The mean ventricular weights of prematurely born infants who had lived for

five or more days were compared with the mean ventricular weights of stillborns of equal crown rump lengths. The left ventricular weights suggest that there has already been some hypertrophy over that which would have occurred *in utero*. The right ventricle growth continued at the same rate as would have occurred had the child remained *in utero*.

### Acknowledgements

Much of the material used in this study came from necropsies carried out through the courtesy of Dr. Warrack. We would also like to thank members of the University Department of Statistics for their help.

Dr. Avinash Mithal is working with a grant from the Medical Research Council.

### References

1. ALIMURUNG, M. N., JOSEPH, L. G., NADAS, A. S. and MASSEL, B. F.: The unipolar precordial and extremity electrocardiograms in normal infants and children. *Circulation*, 4: 420-429, 1951.
2. ASHLEY, L. M.: A determination of the diameters of ventricular myocardial fibres in man and other mammals. *Amer J Anat*, 77: 325-359, 1945.
3. BARCLAY, A. E., FRANKLIN, K. J. and PRICHARD, M. M. L.: The Foetal Circulation. Blackwell, Oxford, 1944.
4. BOELLAARD, von J. W.: Über Umbauvor-  
gänge in der rechten Herzkammerwand während der Neugeborenen, und Säuglingsperiode. *Z. Kreislaufforsch*, 41: 101-111, 1952.
5. DAWES, G. S., MOTT, J. C., WIDDICOMBE, J. G. and WYATT, D. G.: Changes in the lungs of the newborn lamb. *J Physiol*, 121: 141-162, 1953.
6. EMERY, J. L. and MACDONALD, A. M.: The weight of the cardiac ventricles during the later weeks of intrauterine life. *Brit Heart J*, (in press).
7. FULTON, R. M., HUTCHINSON, E. C. and

- MORGAN-JONES, A.: Ventricular weight in cardiac hypertrophy. *Brit Heart J*, 14: 413-420, 1952.
8. HERMANN, G. R. and WILSON, F. N. Ventricular hypertrophy: a comparison of electrocardiographic and post-mortem changes. *Heart*, 9: 91-147, 1922.
  9. HORT, W.: Morphologische Untersuchungen an Herzen vor, während und nach der postnatalen Kreislaufumschaltung. *Virchow Arch Path Anat*, 326: 458-484, 1955.
  10. KEEN, E. N.: The postnatal development of the human cardiac ventricles. *J Anat*, 89: 484-502, 1955.
  11. KEEN, E. N.: Human foetal ventricular outputs. *Nature*, 178: 549-550, 1956.
  12. LEWIS, T.: Observation on ventricular hypertrophy with especial reference to preponderance of one or other chamber. *Heart*, 5: 367-403, 1914.
  13. LIND, J.: Changes in the circulation and lungs at birth. *Acta Paediat (Upps)*, 49: Suppl. 122, 1960.
  14. LIND, J. and WEGELIUS, C.: Angiocardiographic studies on the human foetal circulation. *Pediatrics*, 4: 391-400, 1949.
  15. MERRILD, B. and LIND, J.: Electrocardiographic criteria for enlargement of the right and left ventricles in children under the age of two years. *Acta Paediat (Upps)*, 48: Suppl. 118, 144-146, 1959.
  16. MÜLLER, W.: Die Massenverhältnisse des menschlichen Herzens. Verlag von Leopold, Hamburg and Leipzig, 1883.
  17. PANISELLO, F. S., CASTELLANOS, A., JUNCO, J. A. and VALLADARES, F.: Medidas significativas en el corazón del prematuro. I. Re-evaluación de un método eficaz para determinar el peso real de los ventrículos. II. Estructura anatómica del Eabique interventricular. *Rev Cub Paediat*, 30: 19-34, 35-50, 1958.
  18. VIERORDT, H.: Anatomische Daten und Tabellen, 3rd. ed., Fischer, Jena, 1906.

Received July 12, 1960

Accepted September 16, 1960

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## The Iron and Copper Concentration of the Liver in Intrauterine Life and in Haemolytic Disease

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This investigation was stimulated by the observation that in children dying from haemolytic disease of the newborn, and in some other children, a gross section across the liver, when stained for iron, shows a marked difference in the depth of staining in the different physiological lobes.

Apparently, due to the difference in oxygenation of the supply to the left and right physiological lobes of the liver during intrauterine life (11), the amount of erythropoiesis found in the left liver appears normally to be less than that in the right liver (6). In view of this it seemed likely that there may be a difference in the iron metabolism in the two lobes of the liver, but we have not found any estimations of the liver iron or copper that take into account differences likely to occur as a result of haemopoiesis being different in the left and right livers.

The copper concentration of the liver merited study partly because of its probable relationship with haemoglobin metabolism but also as a parallel metallic substance useful as a control for any changes likely to be found in the iron concentration.

It has long been stated that the iron concentration in the foetal liver is much higher than in the adult liver (8, 21) and

that the copper concentration of the foetal liver is five to ten times that of the adult. (3, 4, 12, 17).

The earlier literature on these metals was summarised by Needham (13) and the more recent literature is summarised in Table 1.

### Material and Method

The material used in this study came from necropsies carried out by the Department of Pathology, The Children's Hospital, Sheffield. The final technique used was that of fixation in 10% formol saline. After fixation the liver was divided into its physiological lobes and thin slices taken for the estimation of the water content. The chemical analyses were carried out on pulverised dried liver. The chemical method used for estimating the non-haemoglobin iron was that described by Ramsay (15) with the modification of carrying out the analysis on dried powdered tissue and omitting the use of carbon monoxide which we found did not significantly alter our results. As an attempt was being made to study the iron concentration of livers from children dying from haemolytic disease and a number of those specimens were only available in a fixed state, the whole of the work was eventually carried out on formalin-fixed material. A considerable amount of time was spent in assessing the errors liable to arise from using

TABLE 1. *Summary of the recent literature on the iron and copper concentration of the liver of the foetus and newborn.*

Author	Number of cases	Age group	Iron		Copper		Comments
			Wet liver mg/100 g	Dry liver mg/100 g	Wet liver mg/100 g	Dry liver mg/100 g	
Gladstone (7)	17	Stillborn, 1 day	9.9-36.8				Non-haemoglobin iron
Schwartz, Baer & Weiser (16)							Similar findings to Gladstone
Ramage, Sheldon & Sheldon (14)	6	Below 24 weeks gestation		210			Total iron
	6	Full term		250			
Toverud (19)	47	Full term		200			
	53	26-38 weeks gestation		168			Method includes some haemoglobin iron
Iob & Swanson (9)	14	Foetuses	19.4-73.7				Estimations on combined liver and spleen
Brückman & Zondek (1)	5	Newborn	8.8-58.5			8.0-38.2	Total iron
Widdowson & Spray (20)	11	Immature and stillborn	6.8-37.0				Non-haemoglobin iron
	14	Immature and stillborn			1.53-8.70		Inorganic iron
Smith, Rosello Say & Yeya (18)	14	0-6 months	6-16				Estimations on combined liver and spleen
Kaldor (10)	6	Stillborn over 3150 g	Over 50				Total iron
	10	Stillborn under 3150 g	Under 50				Non-haemoglobin iron
Morrison & Nash (12)	25	Infants			2.4		
Sheldon & Ramage (17)	5	Foetuses				15-35	
Butt, Nusbaum, Gilmour & Didio (2)	46	Premature				39.5	Includes children up to 3 months after birth
	34	Mature				26.49	

fixed as distinct from fresh livers. It was found that fixed tissues gave slightly lower results.

The copper estimations were carried out on parallel samples using the method of Eden & Green (5).

Each liver was treated as two separate livers, the right and left lobes being treated independently. Thus the total number of specimens examined was 150 from 75 children who had shown no Rh incompatibility and in whom no gross abnormalities of the liver were noted either by naked eye or on examination of sections microscopically. Livers from 11 children who had been suffering from known Rh incompatibility were also examined.

## Results

The general results of the chemical analyses are presented in Table 2. Different aspects of the findings will be discussed separately.

### The Effect of Age

Iron and copper concentrations, given as an average of the left and right lobes, are presented in Fig. 1. Direct inspection of the graphs suggests that there is no difference between the iron and copper concentrations with maturity. This was confirmed on statistical analysis. (The variance ratio (F) for iron being 1.27, for

TABLE 2. Chemical results of the water content and the iron and copper chemical analyses based upon dry weight from the left and right livers of 75 apparently normal livers and 11 children with haemolytic disease. The cases are listed in ascending maturity.

Weeks gest- ation	% Water		Iron		Copper		Weeks gest- ation	% Water		Iron		Copper	
	L	R	L	R	L	R		L	R	L	R	L	R
25	79	78	148	100	21.1	18.5	36	86	88	48	46	11.5	11.3
25	75	74	68	58	7.9	5.6	36	81	81	132	142	21.5	22.6
25	80	81	75	63	16.3	16.8	36	80	80	50	50	8.6	6.7
26	83	82	159	145	17.0	17.0	36	85	85	48	46	8.6	8.0
26	80	80	140	115	16.1	16.4	37	78	79	150	152	11.3	14.9
26	79	79	46	41	15.2	12.2	37	77	75	187	168	16.5	14.1
26	76	77	100	96	11.7	13.5	37	81	79	253	247	19.3	22.0
26	75	77	52	40	10.9	13.2	37	80	81	45	43	20.6	21.8
26	80	80	120	100	11.6	9.5	37	81	77	95	74	13.7	11.7
26	80	79	105	81	23.1	21.8	38	84	84	54	51	4.8	3.8
26	78	77	86	52	10.3	7.9	38	83	80	38	34	6.4	8.2
26	81	80	100	90	14.2	12.9	38	80	80	165	155	19.1	27.3
28	77	75	135	96	16.9	14.7	39	81	82	84	72	23.9	27.8
28	78	78	95	82	16.4	18.0	39	76	75	96	88	14.2	16.8
28	81	82	126	128	10.0	10.2	39	83	83	124	112	14.0	11.2
29	79	78	119	118	20.9	18.5	40	81	81	20	17	26.9	19.4
29	78	79	50	39	13.2	12.9	40	79	78	300	259	13.9	16.6
29	82	81	105	100	15.9	15.8	40	76	75	39	32	22.1	25.1
29	81	80	158	145	12.5	11.3	40	82	82	144	133	6.7	7.5
29	82	81	278	232	15.7	13.4	40	75	74	132	69	18.7	17.0
30	78	78	30	29	35.5	32.0	40	78	78	114	82	5.4	6.1
30	77	76	187	158	15.7	17.1	40	79	80	204	145	12.1	14.6
30	77	75	204	204	20.9	20.9	40	77	77	157	157	8.8	10.6
30	85	83	59	39	—	—	40	76	75	345	252	20.5	23.3
30	82	81	122	121	13.9	11.3	42	78	78	127	77	13.7	11.3
30	84	84	119	106	12.6	12.6	42	78	75	49	38	11.1	9.3
32	81	80	248	190	22.8	17.9	42	80	79	170	181	12.5	15.0
32	78	77	177	165	12.7	14.1	43	84	83	43	55	13.7	11.3
32	78	79	204	162	19.0	19.0	43	77	77	117	109	15.0	19.7
32	81	80	105	85	14.4	12.1	44	80	79	100	119	11.5	13.2
32	82	82	47	40	14.9	18.0	44	84	82	61	69	18.8	22.6
33	80	79	80	81	20.2	14.9	Rh cases						
33	77	77	130	113	21.5	23.0	36	83	84	340	356	17.3	21.7
34	89	90	45	48	6.1	4.8	36	77	77	144	282	7.1	10.7
34	76	76	71	54	16.4	15.7	37	82	81	228	305	17.9	24.9
34	81	84	68	81	17.1	14.7	38	76	75	117	351	11.8	11.5
34	83	83	153	159	9.3	7.6	38	88	88	242	582	18.4	19.0
34	87	86	79	66	24.0	26.2	38	88	88	258	450	16.1	14.0
34	86	85	53	48	8.0	8.8	39	71	74	183	208	8.9	11.6
34	78	77	186	187	25.4	24.2	40	86	86	372	465	8.9	9.8
35	79	79	172	172	21.8	19.3	40	86	85	172	253	5.2	5.8
35	77	77	187	165	22.1	21.8	40	81	81	221	221	5.2	6.0
35	82	84	72	54	10.0	10.0	42	78	78	177	218	8.2	12.7
36	80	80	60	50	27.2	21.8							

$V_1 = 70$ , and  $V_2 = 4$ ,  $F(0.05) = 5.69$ , showing no significant difference between age groups.)

Similar lack of significant difference

was found with the copper estimations. Thus there is no suggestion of any increase in iron or copper concentration with increasing intrauterine maturity.



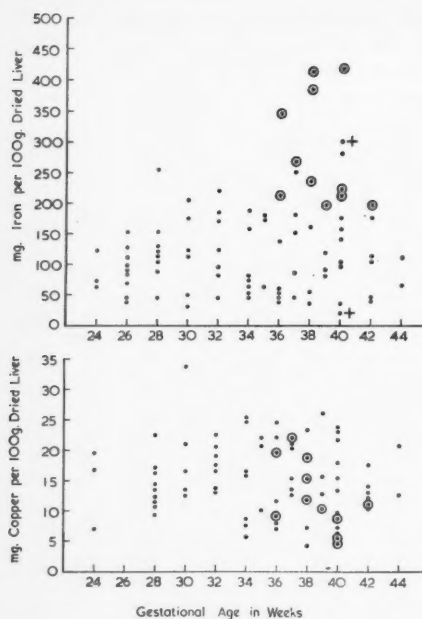


Fig. 1. The iron and copper concentration per hundred grams dried liver related to gestation age in weeks. The average of the two lobes of each case was taken for this chart. Children with haemolytic disease are represented with circles around the significant points.

#### Left and Right Lobes

The iron concentration of the left liver is higher than the right by an average difference of  $2.43 \pm 0.99$  mg/100 g with a 95 % certainty. The difference in iron concentration between the left and the right liver appears to be highly significant, being significant at the 0.1 % level.

Using a similar statistical technique of analysis, it was found that there was no significant difference between the left and right lobes with regard to the copper concentration.

When the effects of age and the left and right lobes were tested for interaction ef-

fect, there was no interaction present. Therefore, it can be concluded that the age and the left and right values are independent for copper and iron.

#### Cases with Haemolytic Disease

The average iron and copper levels of the children with haemolytic disease are presented by large circles around dots in Fig. 1. The iron levels of the Rh cases are considerably higher than the normal range. In these cases, however, the right lobe is higher than the left lobe by an average difference of  $19.00 \pm 12.76$  with a 95 % certainty. This difference is significant at the 1 % level.

When the left and right lobes of the Rh livers are compared with the normal series, there is a very significant difference. Regarding the right lobe, the mean for the Rh cases is 58.09 and for the normal cases 21.96, the standard error of difference being 5.29. Regarding the left lobe, however, the difference is not so great, the mean for the Rh cases being 39.09 and for the normal cases 24.32. The standard error of difference is 5.78. The difference, however, is significant at the 5 % level only, as distinct from the 0.1 % level for the right lobes.

Regarding the copper concentration in the Rh cases there is no significant difference between the left and right lobes. There appears, however, to be a difference between age groups. The mean copper content for the Rh group 36 and under 40 weeks is 15.06 and for the group 40-44 weeks is 7.72, the standard error of difference being 2.748. On this evidence it appears that the difference between the age groups is significant at the 1 % level.

### Summary of Results

Two things emerge from this study concerning the normal series. First, that there is no increase in the normal copper and iron concentration of the liver during later stages of normal intrauterine life. Second, that there is a significant difference between the iron concentration of the left and right livers in normal intrauterine life. The iron concentration of the left lobe is higher than that of the right. There is no difference between the copper concentration of the two physiological lobes.

In children with haemolytic disease there is a very great increase in the iron concentration in the liver. This is present throughout both lobes but is more marked in the right lobe than in the left, so that there is a complete reversion of the apparent physiological ratio of iron concentration. The copper concentration in these cases is significantly diminished only in the children surviving to full term.

### Discussion

Our findings would suggest that the impression gained by some earlier workers that there is an increase in the iron concentration of the liver in later intrauterine life is probably due to the inclusion of some cases of haemolytic disease among their normal series. It is also interesting that in our own material we have found several cases in which the iron concentration was in the same region as those of children with known Rh disease and in whom no serological incompatibility had been found. This suggests to us that these children probably have suffered from some form of haemolytic disease that has not yet been discovered.

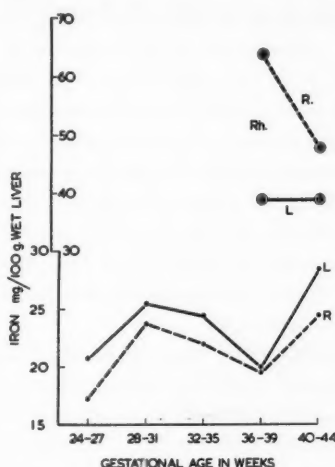


Fig. 2. The average iron concentration in the left and right liver of 75 "normal" livers and 11 livers from children with Rh disease. The latter are represented with circles around the significant points.

The peculiar difference found in the iron in the left and right lobes in the normals and the Rh livers came as a surprise to us (Fig. 2). Under normal circumstances the left physiological lobe has less haemopoietic foci than the right and we have found that the iron content of the left side is higher than the right. This is probably explained by the more rapid turnover of iron in the right than in the left liver. The reverse ratio of concentration occurring in haemolytic disease is more difficult to explain. The amount of haemopoiesis seen in these cases varies very much. It would seem likely, that, in the Rh cases, we see secondary effects due to the child being in a state of chronic relative anoxia. In such a state the left liver is in a favoured position as it has the most highly oxygenated blood, the right liver having perhaps the least oxy-

generated blood in the body. Thus, when toxic and anoxic changes are liable to occur, it is the right liver that suffers rather than the left. Also the right liver is largely supplied with blood that does not go through the placenta and it seems possible that the placenta removes or fixes some of the pre-hepatic iron containing haemoglobin substances and thus shields the left liver from some of the effects of chronic haemolysis.

Regarding the changes in the copper concentration seen in older children dying with haemolytic disease, we are at a loss to explain this unless it could be that there has been a general depletion in copper reserves in the foetus due to the increased turnover of haemoglobin metabolism or perhaps an increased deposition in other organs, possibly the brain.

### Summary

A study of the iron and copper concentration of livers from 75 apparently normal stillborns and 11 infants dying

with haemolytic disease, show that in normal circumstances there is no increase in the iron and copper concentration with increasing intrauterine maturity.

There is no difference between the concentration of copper in the left and right lobes but there is a significant difference in the amount of iron, the left containing more than the right.

In children with haemolytic disease there is a gross increase in the iron concentration of the liver and this is more marked in the right than in the left lobe.

In children with Rh disease there is possibly some diminution in the copper concentration of the liver in those cases that survive to full term.

### Acknowledgement

It is a pleasure to be able to acknowledge the assistance of Miss Doris Kirk of the Sheffield University Department of Statistics, and Dr. A. J. N. Warrack, through whose courtesy many of the necropsies used in the study were available.

### References

1. BRÜCKMANN, G. and ZONDEK, S. G.: Iron, copper and manganese in human organs at various ages. *Biochem J*, 33: 1845-1857, 1939.
2. BUTT, E. M., NUSBAUM, R. E., GILMOUR, T. C. and DIDIO, S. L.: Trace metal patterns in disease states. II. Copper storage diseases, with consideration of juvenile cirrhosis, Wilson's disease, and hepatic copper of the newborn. *Amer J Clin Path*, 30: 479-497, 1958.
3. CARTWRIGHT, G. E.: Copper Metabolism, pp. 274-310. McELROY, W. D. & CLASS, B. (Eds.). Johns Hopkins Press, Baltimore 1950.
4. CUNNINGHAM, I. J.: Some biochemical and physiological aspects of copper in animal nutrition. *Biochem J*, 25: 1267-1294, 1931.
5. EDEN, A. and GREEN, H. H.: Micro-determination of copper in biological material. *Biochem J*, 34 (2): 1202-1208, 1940.
6. EMERY, J. L.: The distribution of haemopoietic foci in the infantile human liver. *J Anat*, 90 (2): 293-297, 1956.
7. GLADSTONE, S. A.: Iron in the liver and in the spleen after destruction of blood and transfusions. *Amer J Dis Child*, 44 (1): 81-105, 1932.
8. HUGOUNENQ, L.: *Compt Rend Soc Biol*, 51: 337, 1899 (cited by NEEDHAM).
9. IOB, V. and SWANSON, W. W.: A study of fetal iron. *J Biol Chem*, 124: 263-263, 1938.
10. KALDOR, I.: Storage iron in the foetus. *At et Ann Med*, 4: 277-278, 1955.
11. MALL, F. P.: A study of the structural unit of the liver. *Amer J Anat*, 5: 227-303, 1906.

12. MORRISON, D. B. and NASH, T. P.: The copper content of infant livers. *J Biol Chem*, 88-89: 479-483, 1930.
13. NEEDHAM, J.: *Chemical Embryology*, 3: 1278, 1931. Cambridge University Press.
14. RAMAGE, H., SHELDON, J. H. and SHELDON, W.: Spectrographic investigation of the metallic content of the liver in childhood. *Proc Roy Soc*, 113B: 308-327, 1933.
15. RAMSAY, W. N. M. and CAMPBELL, E. A.: Iron metabolism in the laying hen. *Biochem J*, 58: 313-317, 1954.
16. SCHWARTZ, BAER and WEISER.: *Z Kinderheilk*, 37: 167, 1924 (cited by GLADSTONE).
17. SHELDON, J. H. and RAMAGE, H.: A spectrographic analysis of human tissues. *Biochem J*, 25: 1608-1627, 1931.
18. SMITH, N. J., ROSELLO, S., SAY, M. B. and YEYA, K.: Iron storage in first five years of life. *Pediatrics*, 16: 166-173, 1955.
19. TOVERUD, K. U.: Investigation on the iron store of newborn infants. *Acta Paediat*, 17: (Suppl. No. 1), 136-139, 1935.
20. WIDDOWSON, E. M. and SPRAY, C. M.: Chemical development in utero. *Arch Dis Child*, 26: 205-214, 1951.
21. ZALESKY: *Z Phys Chem*, 10: 453, 1886 (as cited by NEEDHAM).

Submitted July 12, 1960

Accepted Oct. 4, 1960

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## Chromosomal Abnormality in a Mongolism-Like Syndrome<sup>1</sup>

by J. A. BÖÖK, K.-H. GUSTAVSON and BERTA SANTESSON

The somatic chromosome number in man was unequivocally determined as 46 by Tjio & Levan (13) in 1956. Most patients with mongolism have 47 chromosomes (2, 7, 8). The extra chromosome<sup>2</sup> is morphologically identical with no. 21, i.e. it is a very small acrocentric chromosome with satellites. Preliminary data on meiosis in a few male patients indicate the occurrence of trivalents during the first metaphase (9). Consequently, genuine trisomy is very likely.

This trisomic type of mongolism should be caused by the occasional production of gametes with 24 and 22 chromosomes through non-disjunction during meiosis. The gametes contain either two chromosomes no. 21 or none. The former fertilized by normal gametes give rise to children with mongolism. The fate of zygotes having 45 chromosomes, among which one chromosome no. 21 only, is unknown. This type of monosomy could be lethal at an early fetal stage. The well-known association between mongolism and advanced maternal age indicates that non-

disjunction is more common at oogenesis in women at the end of their reproductive period.

While trisomy for the 21st chromosome is the usual genetical explanation for mongolism, other cytological types have been found. It is not yet clear whether the clinical syndromes associated with these other chromosome abnormalities are identical.

Fraccaro *et al.* (5) found 46 chromosomes in a boy with mongolism. There were five chromosomes of group 19-20 instead of four and only one chromosome 21. The most likely explanation of this karyotype is that the new chromosome originated through centric fusion of two chromosomes no. 21.

Another type of translocation in a girl with mongolism was reported by Polani *et al.* (12). The chromosome number was 46, and the translocation interpreted as having occurred between chromosomes nos. 14 and 21.

The discovery of these translocations also explains the exceptional occurrence of several individuals with mongolism in the same sibship. Two such families have been reported from England (4, 11). In both cases the normal mother had a translocation between chromosomes 15 and 21 and a total of 45 chromosomes.

<sup>1</sup> This investigation is part of a programme aided by grants from the Foundations' Fund for Research in Psychiatry, the Swedish Atomic Research Council, the International Atomic Energy Agency, and the United States Atomic Energy Committee.

<sup>2</sup> The Denver nomenclature of human mitotic chromosomes has been used consistently in this paper (*Lancet* i: 1063-1065, 1960).

The affected children, having a total of 46 chromosomes, apparently had inherited the translocation chromosome 15/21 plus one normal no. 21 from the mother and one normal no. 21 from the father. The common denominator for these non-trisomic types of mongolism is that they should have nearly all the genetical code material of chromosome no. 21 in triplicate. The loss of chromosome material through the translocations is apparently very small.

We have observed a patient with a mongolism-like syndrome who displayed a new and different type of chromosomal aberration which was briefly mentioned in an earlier report (1). This patient is one of a series of 35 individuals with mongolism or suspected mongolism who have been karyotyped in our laboratory since 1959.

### Description of the Patient

S. L. is a girl born Oct. 3, 1956. Her mother, born in 1929, is healthy and S. is her only child. There have not been any abortions. The father, born in 1925, is healthy and has three normal children by a previous marriage. No cases of mongolism, malformations, mental retardation or other significant diseases or defects are known among the near relatives. The mother was healthy during the pregnancy and received regular pre-natal care. The patient was born at full term by an uncomplicated breech presentation. Her weight at birth was 3520 g, her height 47 cm and her head circumference 34 cm. She suckled poorly but no other neonatal complications occurred.

The patient was admitted to a pediatric clinic in Stockholm at the age of 9 months because of constipation and retarded development. She had a slightly reduced muscular tonus and hyperflexible joints. Her height was 64 cm, which is considerably

below the average. Her weight in relation to the height was normal. Her head circumference was 45 cm, which is normal for her age. She had a broad nose bridge, left-sided strabismus, smaller than normal palpebral fissures, bilateral epicanthus, a preauricular outgrowth on the left side and misshapen prominent ears. No physical signs of heart disorder were found.

Roentgenograms of the hip-joints showed both caput femori dislocated above the articular cavity (Fig. 1). Roentgenograms of the skeleton revealed an approximate three months' retardation in the development of the bone nuclei.

The serum cholesterol was 180 mg per 100 ml and the protein bound iodine 7.9  $\mu$ g per 100 ml.

An estimate of her development according to the Bühler-Hetzer scale showed correspondence with a child of seven months, i.e. a retardation of two months.

Ophthalmoscopic examination at the eye clinic of Karolinska sjukhuset in Stockholm revealed a retinal degeneration with scattered white spots in both eye fundi. The optic discs were normal. An electroretinogram showed no pathological changes.

At the age of 15 months the patient was transferred to the orthopedic division of St. Göran's Hospital in Stockholm where the luxations were replaced and casts applied. On account of a continued tendency to dislocation, sub-trochanteric osteotomies were made on both femurs in January, 1959. A further osteotomy was made on the left femur in October, 1959. Since then she has had no dislocations.

### Examinations at the Pediatric Clinic of Karolinska sjukhuset in Stockholm

The patient was first admitted in Oct. 1958 at the age of two years. She has been readmitted several times for follow-up studies. The last time was in Aug. 1960.

Her height has always been below normal. However, her weight in relation to her height has been normal.

Roentgenograms of the head showed a



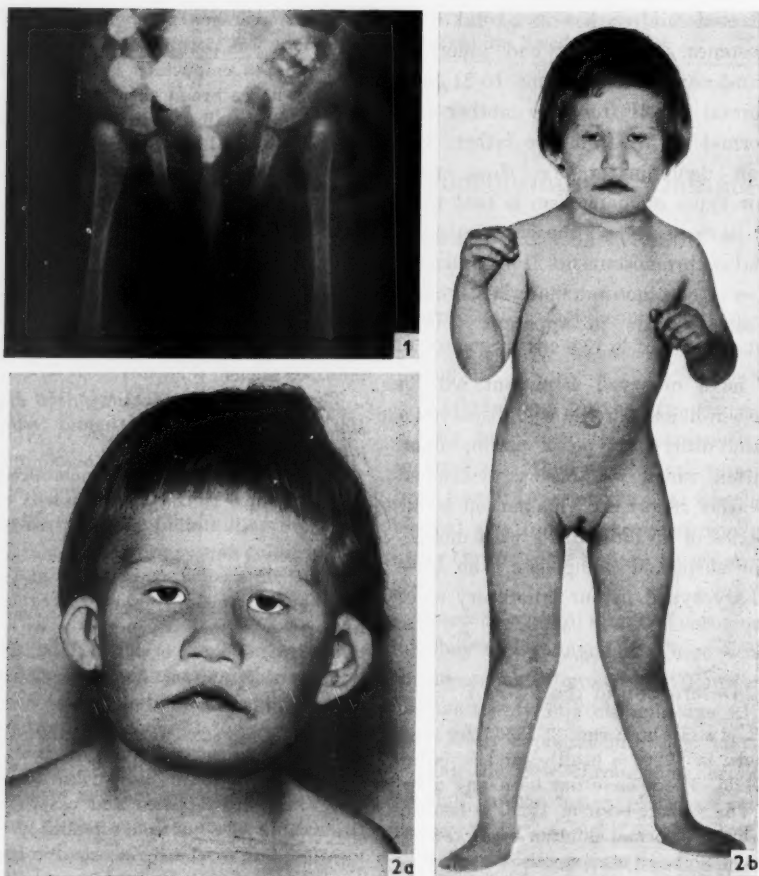


Fig. 1. Roentgenogram of the patient's hip joints.

Fig. 2a and b. The 4 year old girl with a mongolism-like syndrome.

somewhat small calvarium. No intracranial calcifications were visible. There were no deformities of the facial region or of the base of the skull. Roentgenograms of the skeleton showed a normal number of ossification centers with regard to her height but fewer than normal with regard to her age. The bones were slender. No deformities of the middle phalanges of the little fingers were visible. An intravenous urogram showed a

normal anatomy. Roentgenograms of the heart and lungs were normal.

The electrocardiogram and the electroencephalogram were likewise normal. Physiological values were obtained for hemoglobin and red blood cells. The white blood cell count was 5000-7000 per cmm with a normal differential count. The fasting blood sugar was 109 mg per 100 ml, the serum cholesterol 210 mg per 100 ml, the serum

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calcium 10.8 mg per 100 ml, the serum phosphorus 6.1 mg per 100 ml, the serum protein 7.1 g per 100 ml with normal electrophoretic pattern and the protein-bound iodine 9  $\mu$ g per 100 ml.

The Wassermann reaction was negative. A serological test of toxoplasmosis gave a negative result. Direct and indirect Coombs tests were negative. She had no proteinuria. The urinary sediment was normal. Screening of the urine for detection of metabolic disorders (after Lingen, 1960) comprising one and two-dimensional amino acid chromatography,  $\text{FeCl}_3$  reaction and dinitrophenylhydrazin reaction revealed no pathological changes. No sugars indicating metabolic disease were found in the urine.

At 2 years of age the patient was clearly retarded in her development. Judged by fine motor movements her development was equivalent to a child of 15 months, and with regard to gross movements to a child of 11 months. Her social development was equivalent to a child of about 12 months. A further estimate of her development made at the age of 3 years showed that the fine motor movements and the adaptive development were equivalent to that of a child of 2 years. The patient walked at the age of 3 years and 5 months. She could speak single words at the age of 3 years.

An estimate of her intellectual development at 3 years and 7 months of age showed a general level corresponding to a 2½ year old child. The patient has always been easy to handle. She is cheerful and likes to sing and listen to music. Her general health has always been good. Apart from a chronic blepharitis, no infections have been reported.

A physical examination in Aug. 1960 (K.-H. Gustavson) showed a 3 years and 10 months old somewhat small girl with a rather odd appearance (Fig. 2). Her head circumference was 45 cm, the length 15.8 cm, the breadth 13.4 cm and the cephalic index 84. Her height was 88 cm and her weight 13 kg. She had typical epicanthic eye-folds on both sides. The palpebral fissures were narrow and she had a slight blepharitis bilaterally. The nose was flat and relatively small with a

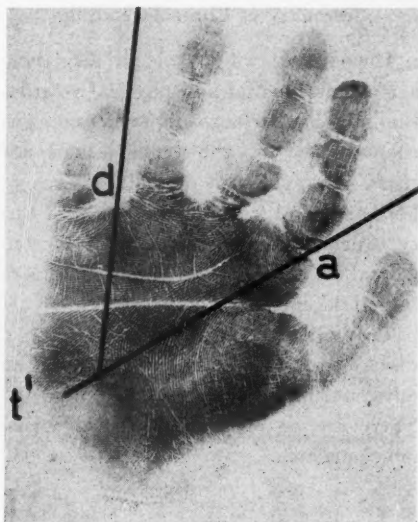


Fig. 3. Dermal pattern of the left hand of the patient. The axial triradius could be classified  $t'$  (maximal  $\text{atd}$  angle  $53^\circ$ ).

broad bridge. The ears were misshapen and prominent. The tongue was of normal size and without sign of papillary hypertrophy or deep furrows, and not protruding. The position and the shape of her teeth were normal, but she had extensive caries and parodontosis. The hands were relatively short and broad with short little fingers. The feet were small and short and in a typical plano-valgus position. The third toe was considerably lower set than the others. The joints were hyperflexible and she had a generally decreased muscular tonus. No physical signs of congenital heart disorder were found. The configuration of the thorax, the abdomen and the external genitalia were all normal. Further routine physical and neurological examination showed no apparent abnormalities. No four-finger line was present.

The dermal ridge patterns of the hands and feet (cf. Fig. 3) were quite different from those usually found in mongolism. We are grateful to Professor L. S. Penrose, who has discussed these details in the appendix.

### Summary of Clinical Findings

The patient is a 4 year old girl displaying a moderate mental and physical retardation. She has certain symptoms and signs characteristic of the mongolism syndrome. However, the dermal patterns of her hands and feet are not typical of this syndrome. She also has a bilateral congenital dislocation of the hips and signs of retinal degeneration.

The etiology is unknown. Thyroid disease is excluded and no evidence of an environmental cause has been disclosed. There are no signs of abnormal sexual development.

### Cytological Observations

Chromosome studies were made according to the technique developed in this laboratory (2, 3, 6). The cytological observations on the patient S.L. were based on 3-5 separate primary cell cultures and their subcultures from one bone marrow biopsy and two skin biopsies taken from the right and the left side of the body. The karyotyping of the parents was based on similar unilateral skin biopsies. The results have been summarized in Table 1.



Fig. 4. Detail of a part of a metaphase plate. The arrows point to 3 satellited acrocentric chromosomes described in the text. Dermal cell culture of the patient.

The material derived from the patient gave sufficiently consistent results to exclude cytogenetical heterogeneity. Among 61 metaphases selected as being apparently undamaged 53 had exactly 46 chromosomes. The deviations shown in Table 1 are the usual ones to be accounted for by methodological errors. In all these 46-chromosome cells we found five short acrocentric chromosomes of group 21-22, i.e. one additional chromosome.

TABLE 1. Summary of chromosome analyses of the patient and her parents.

Individual and origin of cultured cells	Chromosome counts				Total number of analysed cells	Chromosome complement
	45	46	46 ± 1	47		
S. L. bone marrow	1	13	4	1	19	Trisomic 21, monosomic 16, or translocation 16/21
S. L. skin	2	40			42	
Mother, skin		18	1		20	Normal
Father, skin	1	19	1		20	Normal

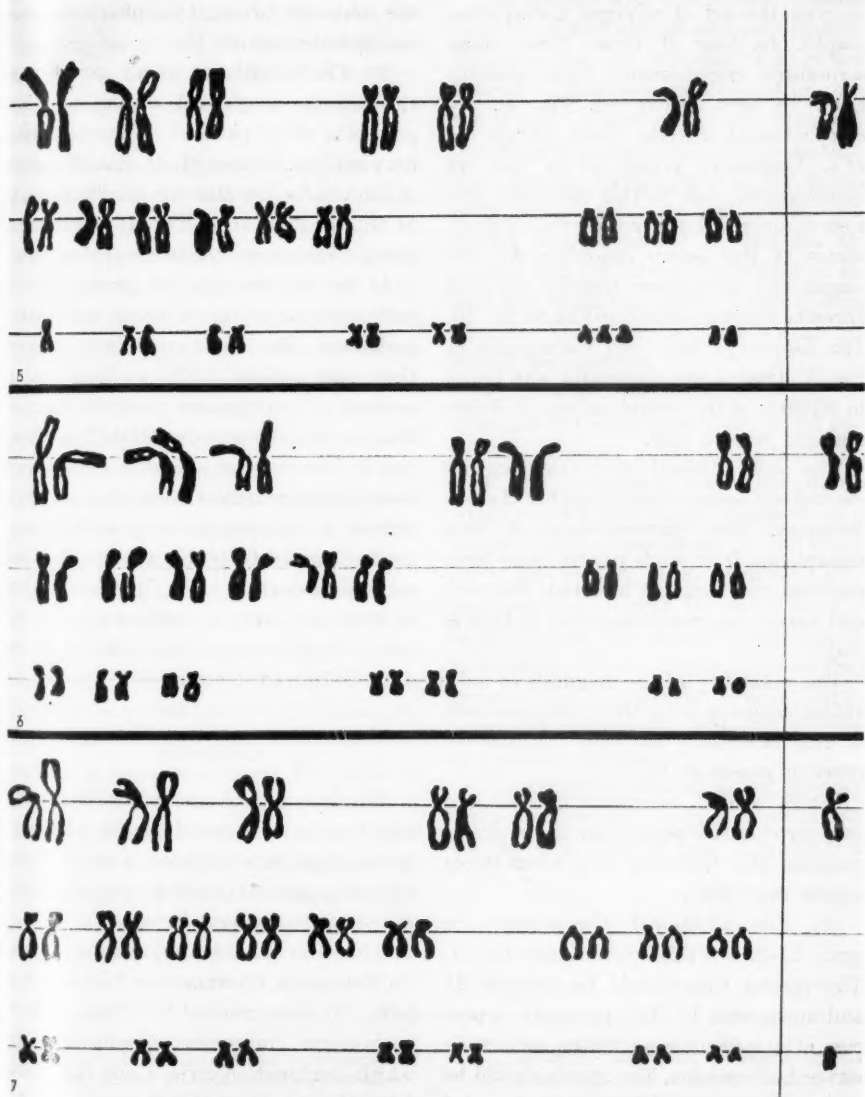


Fig. 5. Chromosome complement of the patient, interpreted as monosomic 16, trisomic 21 or as translocation between the short arms of 16/21.

Fig. 6. Apparently normal chromosome complement of the mother.

Fig. 7. Apparently normal chromosome complement of the father.

Eighteen cells derived from all three biopsies were subjected to a detailed analysis with the aid of enlarged microphotographs. In four of these, three small acrocentric chromosomes with satellites could be seen clearly (cf. Fig. 4). By morphological criteria these should be 21's. Ordinarily group 16-18 has six chromosomes, but in this case only five were recovered. By arranging the chromosomes of this group according to arm length and centromere position the odd chromosome was considered to be no. 16. The karyotype has been exemplified in Fig. 5. Typical sex chromatin was found in 50-60 % of the interphase nuclei of skin and oral mucosa cells.

The cells derived from the parents showed the normal diploid number of chromosomes. The chromosomes of four metaphases from each parent have been matched and analysed in detail. The normal karyotypes are exemplified in Figs. 6 and 7.

The reliability of the diagnosis of individual karyotypes by the techniques used in this laboratory has been discussed in previous papers (3, 6).

While several interpretations of the karyotype of this patient are theoretically possible, the following two alternatives appear most likely:

(1) The additional chromosome in group 21-22 is a third chromosome no. 21. The patient thus should be trisomic 21 and monosomic 16. This postulates a process of non-disjunction during meiosis in one or both parents. The zygote should be the result of a combination of a normal haploid gamete and one gamete lacking chromosome no. 16 and at the same time having two 21's, or a combination of two

gametes one of which had the haploid set plus an additional chromosome no. 21 and the other one having the haploid set minus one chromosome no. 16.

(2) The additional small acrocentric chromosome originated during gametogenesis in either parent by a translocation between nos. 16 and 21. It should consist of the small arms plus the satellites of no. 21 and the short arms of no. 16, assuming a point of exchange close to the centromeres.

At the present time we cannot give a preference to either of these two interpretations. The remarkable fact remains that this patient lacks a considerable amount of chromosome material even if definite monosomy is doubtful. The situation is, however, not unique as this patient from a cytogenetical viewpoint is very similar to another patient with a congenital heart defect who was investigated in this laboratory (3). The alternatives in that case were a combination of trisomy for 19-20 and monosomy for 22 or a 22/19-20 translocation.

### Comments

The diagnosis of mongolism is usually based on the presence of mental deficiency in combination with three or more of the following signs: (1) a brachycephalic skull, (2) oblique palpebral fissures, (3) epicanthic fold over either eye, (4) fissured tongue, (5) blepharitis, (6) transverse fold on either palm, (7) short crooked fifth finger. There is, however, no general agreement as to which combination gives a safe diagnosis. With the discovery of the specific genetic etiology of this syndrome such arguments have become pointless. The question whether or not the term mongolism

should be kept to signify a special clinical syndrome will depend on which differences, mental, physical or biochemical, will be found between patients with different karyotypes. So far it is clear that mongolism does not constitute a clinical and genetical entity.

On the clinical signs and symptoms the patient reported here could be grouped as a case of mongolism. Nevertheless, in as much as this patient is genetically entirely different from the common trisomic type, as well as different from the translocation types so far reported, we are concerned with a new genetical disease entity. From this point of view one might argue that the patient also represents a new clinical syndrome.

Points of particular interest are the occurrence, simultaneously with a number of signs often connected with the mongolism syndrome, of congenital dislocation of the hips and retinal pathology. Neither of these two changes have significant associations with mongolism (10). Their significance in this patient will remain obscure until further cases with the same karyotype have been found. The presence of these two defects may be accidental or due to the expression of recessive genes located in monosomic chromosome segments. In both instances these defects would not form consistent parts of a clinical syndrome.

### Summary

The cytogenetical and clinical observations of a 4 year old girl with a mongolism-like syndrome are reported. The patient showed a moderate mental and physical retardation. Some signs and symptoms considered characteristic of mongolism were present. Others, such as the dermal patterns of hands and feet, rather contradict this diagnosis. In addition the patient had a bilateral dislocation of the hips and unspecific signs of retinal degeneration. There were no signs of abnormal sexual development.

Chromosome studies based on cell cultures derived from bone marrow and skin biopsies revealed a chromosome number of 46 and a consistent karyotype pattern which was interpreted as a combination of trisomy for chromosome no. 21 and monosomy for no. 16, or alternatively as a translocation between the short arms of 16/21. Typical sex chromatin was present in 50-60% of the interphase nuclei. Both parents had apparently normal karyotypes and were free from signs or symptoms of significant disease.

This patient represents a new genetical disease entity and clinically a variant of the common trisomic type of mongolism.

### References

1. BÖK, J. A.: Mongolism and other conditions associated with chromosomal variations. *Acta Soc Med Upsal*, 65: 1-2, 1960.
2. BÖK, J. A., FRACCARO, M. and LINDSTEN, J.: Cytogenetical observations in mongolism. *Acta Paediat (Upps)*, 48: 453-468, 1959.
3. BÖK, J. A., SANTESSON, B. and ZETTERQVIST, P.: Association between congenital heart malformation and chromosomal variations. *Acta Paediat (Upps)* 50: 217, 1961.
4. CARTER, C. O., HAMERTON, J. L., POLANI, P. E., GUNALP, A. and WELLER, S. D. V.: Chromosome translocation as a cause of familial mongolism. *Lancet* ii: 678-680, 1960.

5. FRACCARO, M., KALJSER, K. and LINDSTEN, J.: Chromosomal abnormalities in father and mongol child. *Lancet*, *i*: 724-727, 1960.
6. — Somatic chromosome complement in continuously cultured cells of two individuals with gonadal dysgenesis. *Ann Hum Genet (Lond)*, *24*: 45-61, 1960.
7. JACOBS, P. A., BAIKIE, A. G., BROWN, W. M. C. and STRONG, J. A.: The somatic chromosomes in mongolism. *Lancet*, *i*: 710, 1959.
8. LEJEUNE, J., GAUTIER, M. and TURPIN, R.: Étude des chromosomes somatiques de neuf enfants mongoliens. *C R Acad Sci (Paris)*, *248*: 1721-1722, 1959.
9. MILLER, O. J., MITTWOCH, U. and PENROSE, L. S.: Spermatogenesis in man with special reference to aneuploidy. *Heredity*, *14*: 456, 1960.
10. ØSTER, J.: "Mongolism". *Opera ex domo Biol Hered*, Vol 32 (Copenhagen) 1953.
11. PENROSE, L. S., ELLIS, J. R. and DELHANTY, J. D. A.: Chromosomal translocations in mongolism and in normal relatives. *Lancet*, *ii*: 409-410, 1960.
12. POLANI, P. E., BRIGGS, J. H., FORD, C. E., CLARKE, C. M. and BERG, J. M.: A mongol girl with 46 chromosomes. *Lancet*, *i*: 721-724, 1960.
13. TJO, J. H. and LEVAN, A.: The chromosome number of man. *Hereditas*, *42*: 1-6, 1956.

Submitted Jan. 24, 1961

Accepted Jan. 30, 1961

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## Appendix

The following analysis of dermal ridge patterns was made by Professor L. S. Penrose, at the Galton Laboratory, London W.C. 1.

### Finger tip patterns

Left: V - vestigial whorl (2); IV - arch (0); III - arch (0); II - arch (0); I - ulnar loop (7).

Right: V - whorl (9 or 6); IV - arch (0); III - arch (0); II - arch (0); I - ulnar loop (9).

Total ridge count (Q) = 27

### Palmar patterns

Left: The *a* triradius is duplicated; *b* and *c* triradii are fused in a way which occurs in syndactyly of 3rd and 4th digits. The axial triradius could be classified *t'*. (Maximal *atl* angle 53°.) There is a well-defined thenar-first interdigital pattern.

Right: The *a* triradius is duplicated; *b* and *c* are fused as on the left hand. The axial triradius is in the position *t''* (maximal *atl* angle 61°) and there is a subsidiary triradius on the extreme ulnar aspect. A small thenar-first interdigital pattern is present.

### Plantar patterns

Left: The hallual area contains a whorl and there is another whorl in the third-fourth interdigital region.

Right: The hallual area contains a well-marked whorl and there is a digital loop between the hallux and second toes.

### Note

The configurations on the fingers, palms and soles do not suggest the diagnosis of mongolism, indeed they practically exclude it. The finger print patterns show 6 arches out of 10 which would be a very unusual finding in mongolism. Moreover, bilateral thenar patterns are very rare and hallual whorls also are most uncommon in mongolism.

The arrangement of the main lines in upper part of the palm is peculiar but again not characteristic of mongol hands. On the contrary, it is of a type found on some hands of females with gonadal agenesis (Turner's syndrome); the presence of rather wide *atl* angles together with thenar-first interdigital patterns I have also found to be characteristic of that condition.

## Reference

- WALKER, N. F.: The use of dermal configurations in the diagnosis of mongolism. *J Pediatr*, *50*: 19-29, 1957.



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## Exudative Enteropathy

by L. J. DOOREN, H. H. VAN GELDEREN and C. L. J. VINK

Hypoproteinaemia is not an uncommon finding in many diseases. Usually a specific cause for the low serum protein can be found. After careful analysis, however, there remains a group of patients in whom the cause of hypoproteinaemia cannot be identified. This disease has been called essential or idiopathic hypoproteinaemia. The main symptoms of essential hypoproteinaemia are oedema, disturbance of growth and, very often, gastrointestinal complaints (3, 5, 9-11, 13, 15, 20-22, 25-27, 31, 32, 34, 37-39, 43, 47). Often there is steatorrhoea, sometimes hypertrophic gastritis. Blood analysis reveals hypoproteinaemia; usually the albumin fraction is decreased but sometimes one or all of the globulin fractions are reduced. Hypocalcaemia is frequent. The intake and resorption of proteins is normal, as is liver, pancreatic and renal function. The course of the disease can be mild or transient (especially in infancy), but is often chronic and sometimes fatal.

A deficiency of protein synthesis was first postulated as the cause of the hypoproteinaemia, but since 1949 various workers, using different methods of labeling proteins or amino-acids, have shown a greatly accelerated disappearance rate of serum-albumin and often also of gamma-

globulin (1, 2, 5, 7, 16, 21, 26, 28, 36, 39, 40, 42, 44). It was assumed from these investigations that the hypoproteinaemia was the result of hypercatabolism, as no loss of protein could be found.

Though diarrhoea and fatty stools are often encountered in the published case-histories of essential hypoproteinaemia, the cause of the low serum protein was not sought for in intestinal disease until recently. However, in 1959 Citrin (6) noted the frequent occurrence of hypoproteinaemia in hypertrophic gastritis. Radioactive labeled albumin was injected intravenously into a patient with hypertrophic gastritis and was recovered from the gastric contents from which Citrin deduced that the hypoproteinaemia in this patient was caused by loss of protein into the stomach. Gordon (18, 19) suggested that in essential hypoproteinaemia leakage of proteins into the intestines could explain both the low serum-protein and the rapid disappearance of injected albumin or gammaglobulin. The proteins lost into the intestinal lumen will be reabsorbed after being split into amino-acids and resynthesized to serum proteins, after which part of them will again be lost into the intestines. After maximal protein synthesis has been reached hypoproteinaemia will develop.



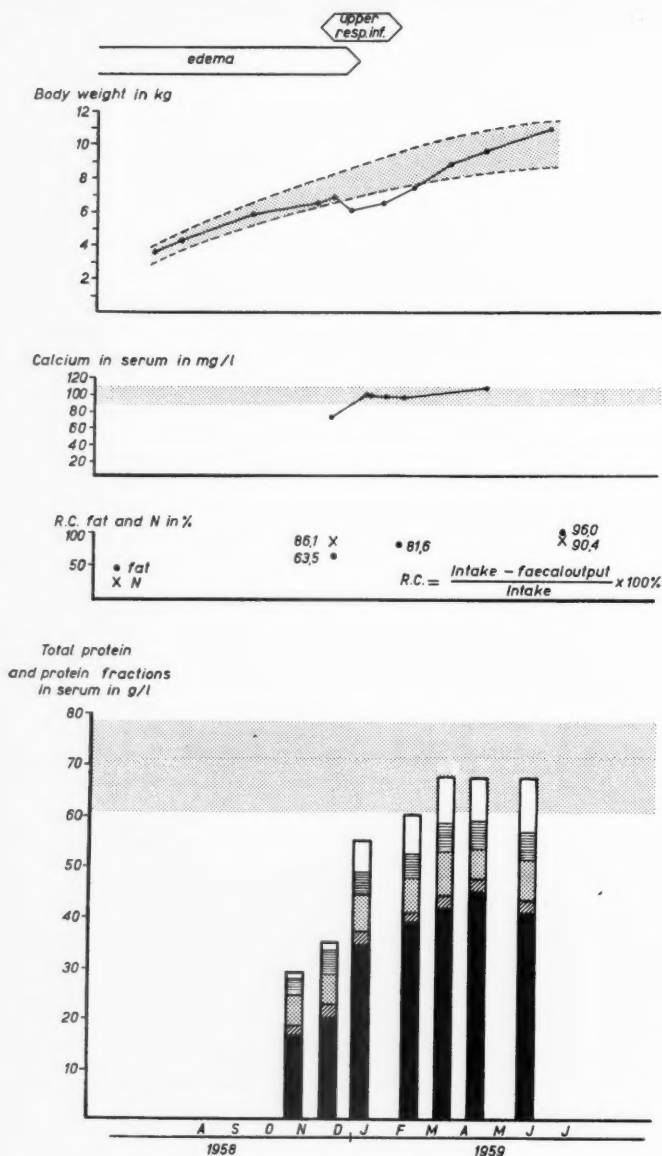


Fig. 1.

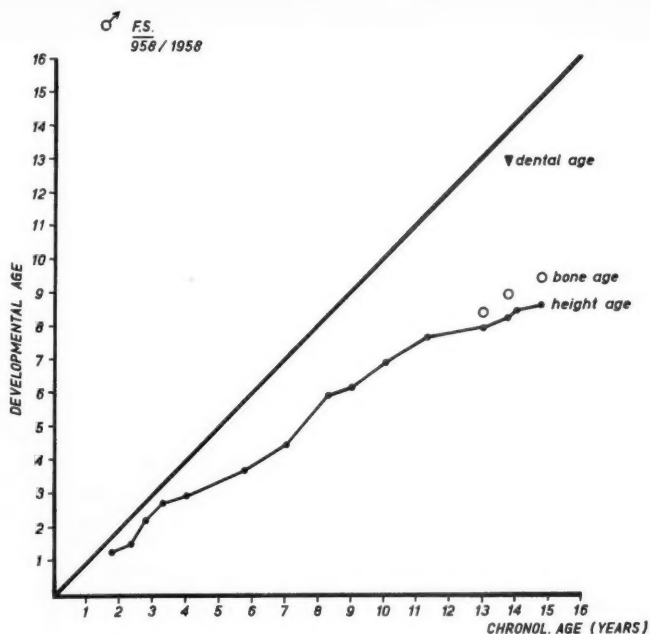


Fig. 2.

No loss of proteins with the stools can be demonstrated and nitrogen resorption will be normal. Gordon demonstrated this leakage by the intravenous injection of radioactive labeled polyvinylpyrrolidone (PVP); this compound, which has an average particle size somewhat lower than that of serum albumin, cannot be metabolized after leakage into the intestines.

In 9 patients with essential hypoproteinaemia, Gordon found a much larger part of the injected PVP in the stools than in 41 normal controls. He suggested the name "exudative enteropathy" for these cases of hypoproteinaemia (19).

Since Gordon's first experiments, other cases of essential hypoproteinaemia have

been published in which also an increased loss of injected labeled PVP with the stools was reported.

#### Case Histories

*Patient A.*, a female infant, developed hypoproteinaemia at the age of four months. Serum protein values: 30 g/L total protein, of which albumin 59.9%,  $\alpha_1$ -globulin 7.3%,  $\alpha_2$ -globulin 17.4%,  $\beta$ -globulin 12.1%,  $\gamma$ -globulin 3.3%. Serum calcium was 77 mg/L, intake and resorption of protein was normal. There was no albuminuria but there was moderate hyperaminoaciduria with a normal pattern. Fat resorption coefficient was only 64% both on glutenfree and normal diet. Liver, pancreatic and renal functions were normal. No PVP test was performed in this patient.

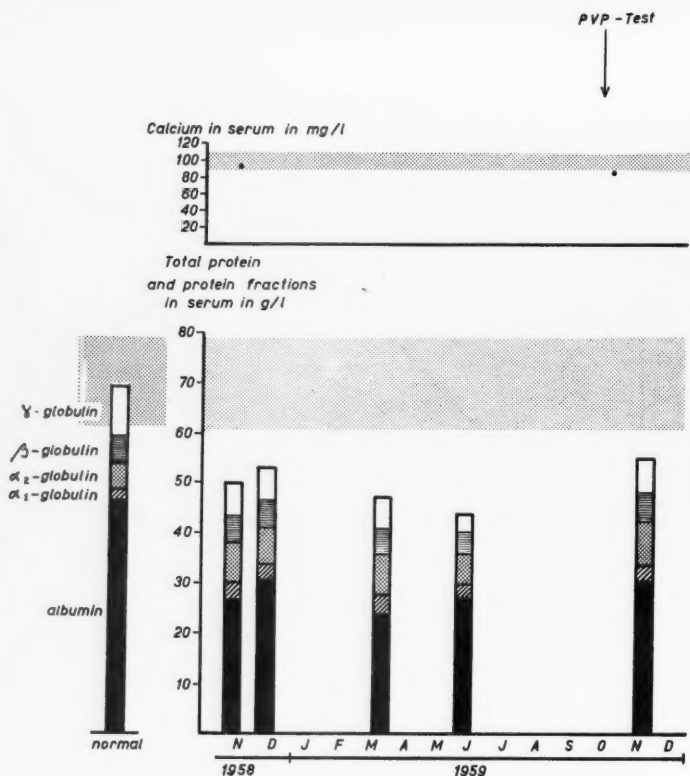


Fig. 3.

The diagnosis was essential hypoproteinaemia. Figure 1 shows the course of the disease. The child recovered spontaneously; the return to normal of serum proteins was accompanied by an increase of fat resorption to normal values, indicating a relationship between the two phenomena. Aminoaciduria also became normal. She is an example of the transient infantile form of essential hypoproteinaemia (4, 12).

*Patient B.*, a boy of 13 years of age, was investigated because of stunted growth with mental deficiency (IQ: 63), for which no specific cause could be found (Fig. 2). Hypoproteinaemia (49 g/L) (Fig. 3) was an incidental finding since there were no clinical symptoms. Thorough investigation did not

disclose a cause for the low serum proteins. Serum calcium, fat-resorption and aminoaciduria were normal. A diagnosis of essential hypoproteinaemia was made.

The boy suffered from stomach-cramps; a year before a duodenal ulcer was found (Dr. Huysinga) and at the time of our study there was X-ray evidence of duodenitis and jejunitis (Fig. 4). A PVP test revealed an excessive loss of PVP with the stools.

*Patient C.*, a girl of 14 years of age, had generalized oedema since birth (Fig. 5). There was also chronic diarrhoea. Mental and somatic development were retarded (Fig. 6). The main biochemical data are summarized in Fig. 7. As no cause for the hypoproteinaemia was found, the diagnosis

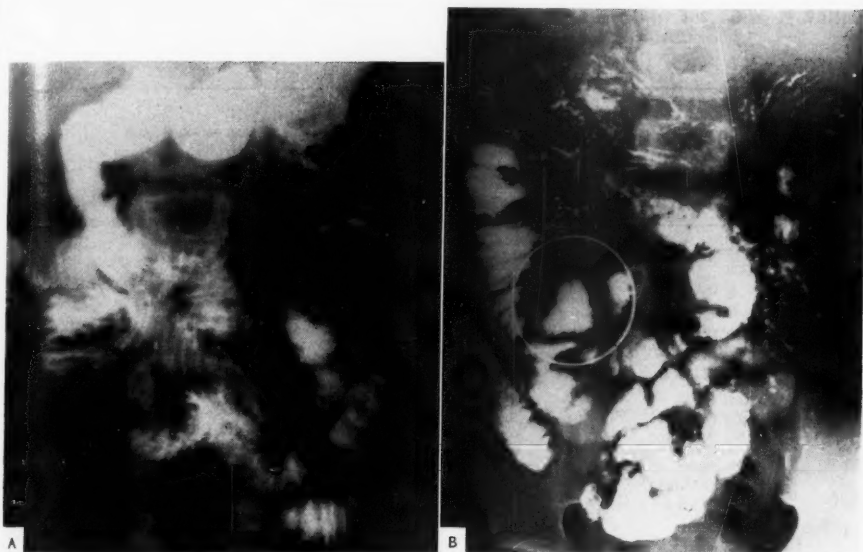


Fig. 4. Roentgenological findings in patient B.—A. The duodenum descendens has lost its mucosal pattern. The contours are coarse, and the duodenum is partly widened. B. The jejunum has also a coarse mucosal pattern and contains rather much secretion. Segmentation and scattering of the barium contrast is noted.

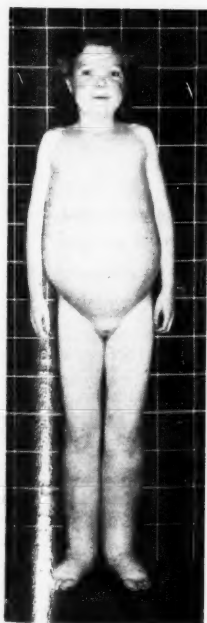


Fig. 5.

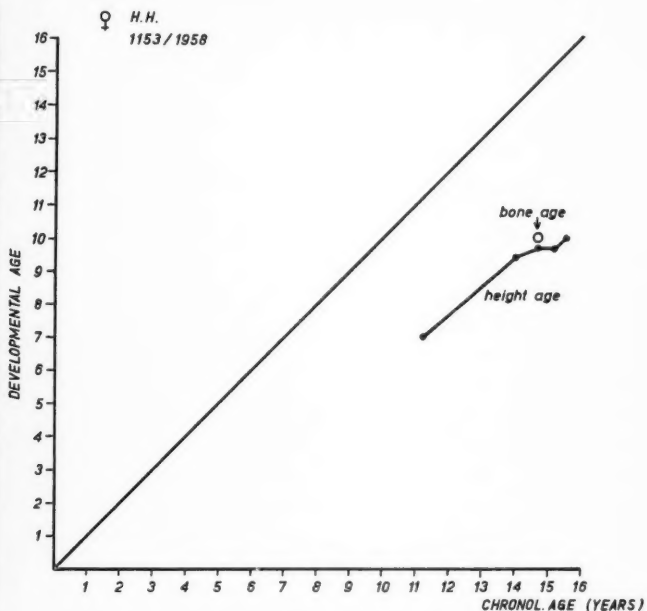


Fig. 6.

TABLE 1. *R.C. fat and R.C. nitrogen, nitrogen balances and aminocacid excretion in patient C.*

Balance periods	Diet	Mean daily urinary nitrogen excretion in g	Mean daily faecal nitrogen excretion in g	Free $\alpha$ -amino-N in urine in % of total urinary nitrogen excretion	R.C. nitrogen in %	R.C. fat in %	$\Delta N \pm \sigma$ in g/day
15.10-20.10 1958 5 days	Gluten + 81 g fat/day					80	
6.12-12.12 1958 6 days	Gluten free 42.1 g fat/day 7.7 g N/day		1.18		84.6	88.5	
11.1-17.1 1959 6 days	Gluten + 57 g fat/day 10.75 g N/day	7.38	1.09	0.8	89.8	86.5	$+ 2.28 \pm 0.3$
17.1-23.1 1959 6 days	Gluten + 57 g fat/day 10.75 g N/day	8.26	2.15	0.8	80.0		$+ 0.34 \pm 0.3$
23.1-29.1 1959 6 days	Gluten + 57 g fat/day 10.75 g N/day	7.56	1.47	1.0	86.5		$+ 1.72 \pm 0.3$
29.1-4.2 1959 6 days	Gluten + 57 g fat/day 10.75 g N/day	8.51	2.02	0.8	81.2		$+ 0.22 \pm 0.3$
4.2-10.2 1959 6 days	Gluten + 57 g fat/day 10.75 g N/day	8.55	1.55	1.0	85.6	86.5	$+ 0.65 \pm 0.3$
Normal values				< 1.4 %	85-95 %	$\geq 95$ %	

$$R.C. = \frac{\text{Intake} - \text{Faecal output}}{\text{Intake}} \times 100 \%$$

$\Delta N/\text{day}$  = Daily intake - daily (urinary + faecal) output.

The balance data have been corrected for systematic losses in the collection of urine and faeces.  $\sigma$  has been estimated for random errors in collection, weighing, and chemical analysis of the samples.

was essential hypoproteinaemia. Table 1 shows the results of balance studies. Nitrogen balance seemed to be nearly in equilibrium (normal loss with nails, hair etc. being about 1 g a day (30)), but rather frequent abdominal punctures were necessary, leading to much loss of protein (Table 2) and therefore a negative nitrogen balance.

X-ray studies did not disclose marked abnormalities of the gastro-intestinal tract.

In November 1959 a PVP test revealed an abnormal loss of PVP with the stools (Table 3).

### The PVP Test

In patients B and C a PVP test was performed as described by Gordon. A quantity of  $^{131}\text{I}$ -PVP was sent to us by Dr R. S. Gordon, Jr., from the National

in

N ± 6  
g day

28 ± 0.5

34 ± 0.5

2 ± 0.5

2 ± 0.5

5 ± 0.5

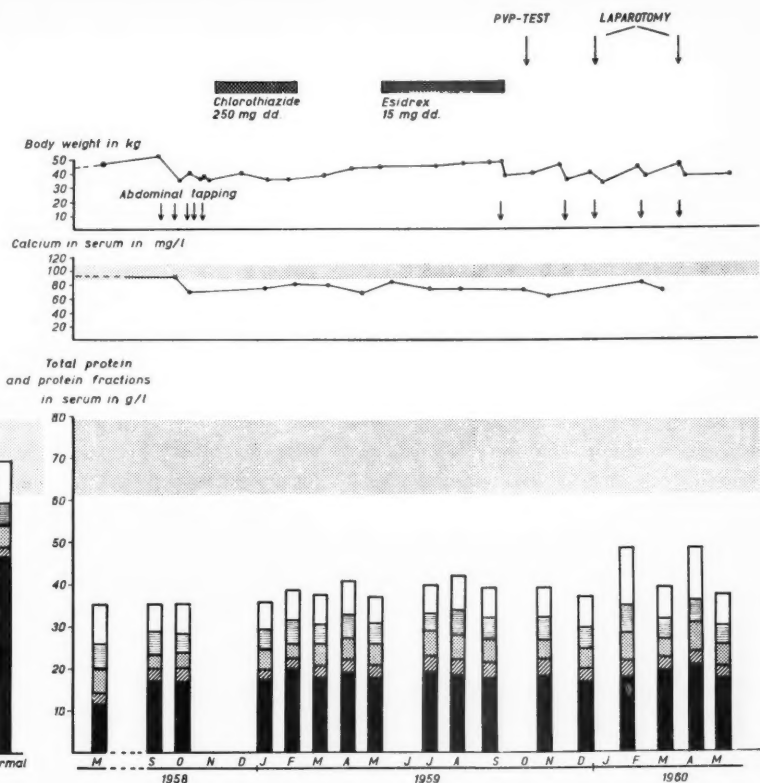
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Fig. 7.

Institutes of Health, Bethesda, Md., U.S.A. As a control a PVP test was also performed in a mentally deficient child, convalescent from an acute infection (control). Excretion of PVP via the faeces in this child was in the normal range.

The radioactivity of the solution was determined after dialysing and sterilizing. A quantity, corresponding to about 0.40  $\mu$ C of  $I^{131}$ -PVP per kg body weight was injected intravenously without side-effects. The thyroid gland was blocked by oral doses of Lugol's solution. The radio-

activity of all urine and faeces samples was measured for 7 days, and the disappearance rate in plasma was determined. The contents of the stomach and duodenum were aspirated in the first hours after the injection of PVP, and radioactivity was measured. The results are shown in Table 3. The loss of PVP with the stools was abnormally high in both patients. Radioactivity in the contents of stomach and duodenum did not vary systematically. A large part of the PVP remained in the body for many days; this retention was higher in the

TABLE 2. *Composition of diet*

Date	9.9 1958	1.10 1958	13.10 1958	21.10 1958	30.10 1958
Diet			81 g fat/day	81 g fat/day	81 g fat/day
Volume in ml	11,200	7000	100	460	3000
Colour	milky	milky	milky	milky	milky
Total protein in g/l	20			15	16.5
Total protein loss in g	224			72	49.5
Total lipid in mg/100 ml	500		513	420	200
Cholesterol in mg/100 ml	78		61	68	62

patients B and C than in the control subject. The same phenomenon was found in the study of Schwartz (39). From the limited number of observations it is difficult to conclude that this is a real difference. The result is possibly due to a higher renal clearance of  $I^{131}$ -PVP during the first day of the test caused by a higher average blood level of this substance in the control than in the patients B and C. (Compare also:  $t_{1/2}$ -values in Table 3.) However, several other hypotheses are possible.

Young (47) demonstrated that 77 % of the injected  $I^{131}$ -PVP was retained in the body in his patient after 3 days, mostly

in the liver, which quantity further decreased by about 1 % a day.

Fig. 8 shows the results of all PVP tests published up to November 1959. The data for our patients lie well outside the normal range. A more extensive and technical description of the PVP-test in our patients had been published elsewhere (45).

Since the outlook for patient C was poor, and both steatorrhoea and the PVP-test results suggested intestinal abnormalities or disease, it was decided to perform a laparotomy. It has been shown (21, 39) that in some cases of essential hypoproteinaemia surgical intervention can lead to cure. The operation was performed on

TABLE 3. *The cumulative excretion (E) in urine (U) and faeces (F) and the retention (100 minus E) of  $I^{131}$ -PVP, calculated in percents of injected dose, respectively after 4 and 7 days after injection. The disappearance rate of  $I^{131}$ -PVP from the plasma is given as  $t_1$ .*

Time after injection of $I^{131}$ -PVP	Idiopathic hypoproteinaemia						Oligophrenia		
	Patient B			Patient C			Control		
	Excretion			Excretion			Excretion		
	U	F	Retention	U	F	Retention	U	F	Retention
4 days	12.0	4.0	84.0	14.1	4.0	81.9	28.7	1.2	70.
7 days	13.7	4.3	82.0	15.5	4.5	80.0	30.3	1.8	67.9
$t_1$ between 0 and 24 hours after injection	5.2 Hours			8.7 Hours			13.1 Hours		



on patient C.

1.10.58	10.10.58	16.9.1959	2.11.1959	7.1.1960 Laparotomy	24.2.1960	11.4.1960 Laparotomy
51 g fat/day	51 g fat/day	11,000	55 g fat/day		53 g fat/day	53 g fat/day
460 3000		8400	8400	5800	6700	± 6000 cc
milky	milky	milky	milky	milky	yellowish turbid	yellowish turbid
15 16.5		17	17	19	31	18
72 49.5		142.8	142.8	110.2	207.7	± 108
420 200		264	264	232	104	
68 62		74	74	94	87	

de- January 7, 1960. The small intestine showed a much thickened wall with increased vascularity and a purplish-red colour. In the distal 100 cm these signs were less pronounced. The omentum was reduced to a coarse mesh, and there was no omental bursa (Fig. 9). The stomach, duodenum, and large intestine had a normal appearance.

Biopsies of the small intestine at several levels revealed remarkable microscopic changes.

The mucosa was swollen and chronically infiltrated. The submucosa was oedematous, showing large histiocytes with foamy protoplasm (Fig. 10). The number of these macrophages increased in distal direction and reached a maximum in the second half of the jejunum and the beginning of the ileum. In the muscular layer a yellow-brown pigment was seen, increasing in quantity in the distal direction. The greatest concentration of the pigment was found in the distal part of the ileum. The

Frequency-distribution of the percentual PVP-excretion with the faeces in 52 controls and 17 patients with idiopathic hypoproteinaemia.

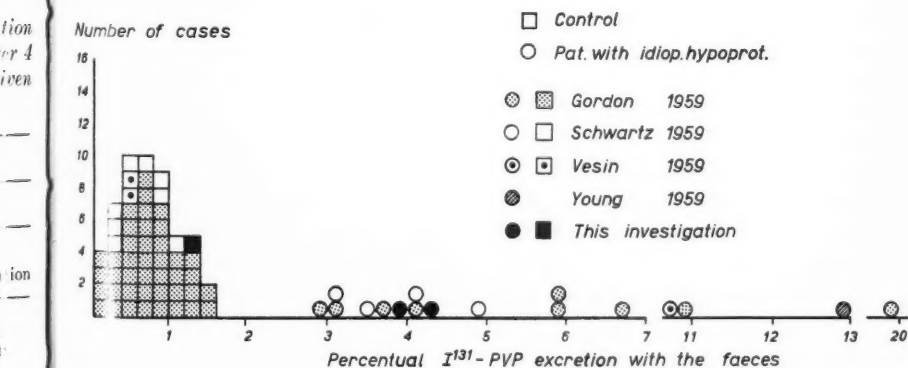


Fig. 3. Frequency-distribution of the percentual PVP-excretion with the faeces in 52 controls and 17 patients with idiopathic hypoproteinaemia.

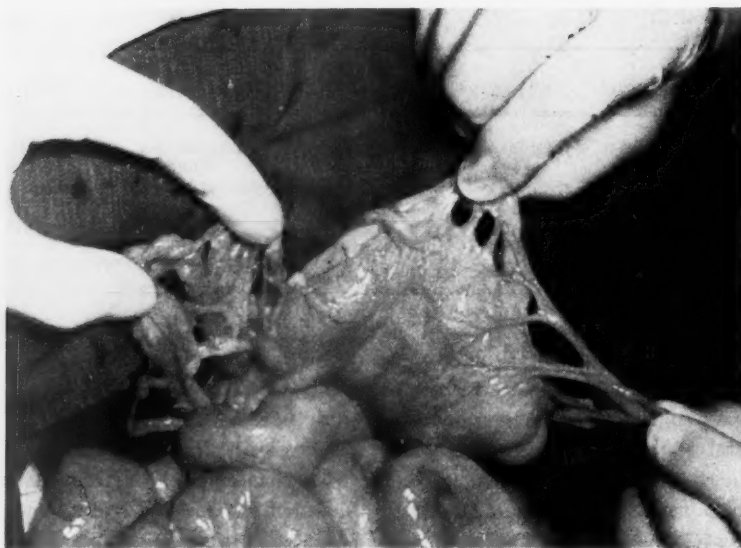


Fig. 9.

mesenteric lymphnodes also contained large quantities of this pigment. Extensive histochemical investigation showed that these macrophages contained protein-like substances and no fat. The pigment could be classified as a lipofuscin.

The biopsies of stomach, large intestine, and liver showed no abnormalities except oedema.

The most probable explanation for these pathological findings seemed to us to lie in a direct relationship between the large cells in the submucosa and the loss of protein. It was assumed that these cells might take up proteins leaking from the vessels into the intestinal lumen. The pigment was considered to be a secondary phenomenon. It has been described in other cases of hypoproteinaemia (35), but in these cases it was not confined to the

small intestine. If this theory was correct, the main "protein-leaking" part of the small intestine would be the part where most macrophages were found. We therefore decided to perform a resection of this part.

The patient recovered without complication from the first operation. On April 11, 1960 a second laparotomy was performed. Twenty-four hours and again one hour before operation small doses of human serum albumin labeled with fluorescein were injected intravenously. During the operation biopsy samples were again taken from the small intestine at several levels. Frozen tissue slices were prepared and studied both with a fluorescence-microscope and after haematoxylin-eosin staining. The findings were the same as at the first operation. The fluorescein-labeled

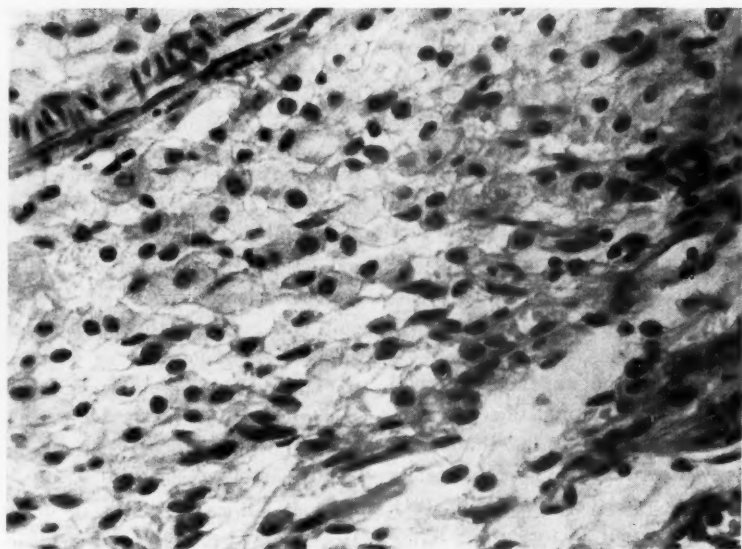


Fig. 10.

albumin was found in large quantities in the submucosal macrophages.<sup>1</sup> The part of the small intestine with the largest number of these pathological cells, according to both microscopical methods, was resected: 30 cm distally from the ligament of Treitz up to 230 cm from the ligament of Treitz.

The patient's recovery from the operation was quick and uneventful. During a short period after both operations a slight increase in total serum protein was noted. As Figure 7 shows, this effect is mainly due to a rise in plasma globulins, especially gamma globulin, and not of plasma albumin. One month after both

operations, the serum protein level was as low as before surgery. The resection has not cured our patient. In all probability, the remaining intestine is too severely diseased.

#### Discussion

Our first patient showed a close relationship between hypoproteinaemia and steatorrhoea. Nevertheless, pancreatic function, gluten-tolerance, and nitrogen resorption were normal, so that steatorrhoea was not the cause of the hypoproteinaemia. This suggests an intestinal lesion causing both symptoms. The second patient had hypoproteinaemia, normal nitrogen resorption, roentgenological duodenitis and jejunitis, and a pathological PVP-test. The third patient showed all the clinical symptoms of essential hypoproteinaemia

<sup>1</sup> We thank Dr. R. O. v. d. Heul and Dr. J. Oord of the Department of Pathology, University Hospital, Leyden, for their histological and histochemical examination and for suggesting and carrying out the method of fluorescein labeling of human serum albumin.

with steatorrhoea, a pathological PVP-test and distinct macroscopical and microscopical lesions of the small intestine at laparotomy.

Taken together, the findings in these three patients are a strong argument for the theory that many patients with an unexplained hypoproteinaemia are in fact suffering from an exudative enteropathy (Gordon). In some cases, this diagnosis may lead to surgical intervention and cure (21, 39).

It is possible that in many normal individuals a small amount of serum protein leaks into the intestinal lumen; in patients with essential hypoproteinaemia this leakage exceeds the normal limits (21). The most important factor in the hypoproteinaemia in these cases is the hypoalbuminaemia, probably because of the small molecular size of plasma albumin as compared with plasma globulins. The hypocalcaemia can be explained by the decrease of the protein-bound fraction of serum calcium (23, 29, 41).

The exact nature and mechanism of protein loss into the diseased intestine is not yet known. Pathological and histochemical studies of the biopsies after in-

jection of fluorescein-labeled albumin may teach us more.

### Summary

Three patients with essential hypoproteinaemia are described. In all of them, signs and symptoms suggested an intestinal lesion. In two of them a PVP test was performed and gave a pathological result. Our cases and those of others strongly support the hypothesis that many cases of unexplained hypoproteinaemia are caused by intestinal disease. The poor general condition of one of the patients led us to perform a laparotomy. Biopsies showed distinct lesions of the small intestine. A large part of the diseased small intestine was resected without benefit.

In some chronic cases surgical intervention may be tried. The PVP test, as first described by Gordon, is of great diagnostic aid in such cases.

### Acknowledgement

We wish to thank Dr R. S. Gordon, Jr. for his generous provision of  $I^{131}$ -PVP.

### References

1. ALBRIGHT, F., BARTTER, F. C. and FORBES, A. P.: The fate of human serum albumin administered intravenously to a patient with idiopathic hypoalbuminemia and hypoglobulinemia. *Trans Ass Amer Physcns*, 62: 204, 1942.
2. BENDICH, A. and KABAT, E. A.: Immunochemical estimation of the rate of disappearance of transfused gammaglobulin from the blood in two cases of hypoproteinemia. *J Lab Clin Med*, 34: 1066, 1949.
3. BINGER, M. W. and KEITH, N. M.: General edema of indeterminate etiology. *JAMA*, 109: 1, 1937.
4. BOUND, J. P. and HACKETT, W. R.: Idiopathic hypoproteinaemic oedema and aminoaciduria in an infant. *Arch Dis Child*, 28: 104, 1953.
5. CATTELL, W. R. and NORRIS, T.: Idiopathic hypercatabolic hypoproteinaemia. *Proc Roy Soc Med*, 50: 514, 1957.
6. CITRIN, Y., STERLING, K. and HALSTEAD, A.: The mechanism of hypoproteinemia associated with giant hypertrophy of the gastric mucosa. *New Engl J Med*, 257: 906, 1957.
7. CORBEEL, L., MALBRAIN, H. and VISSCHER, DE M.: L'hypoprotéinémie essentielle chez l'enfant. *Acta Paediat Belg*, 8: 337, 1954.

8. CSEPAL, K.: Über isolierte Störung des Salzstoffwechsels bei einem Fall von polyglandulärer Sklerose. *Klin Wschr*, 2: 1988, 1923.
9. DEBRÉ, R., ROYER, P., LESTRADET, H. and REISER, D.: L'hypoprotéinémie idiopathique de l'enfant. *Sem Hôp Paris*, 29: 1899, 1953.
10. DEGNAN, T. J.: Idiopathic hypoproteinemia. *J Pediatrics*, 51: 448, 1957.
11. DELAITRE, FONTY: Un cas d'hypoprotéinémie d'apparence essentielle avec hypogammaglobulinémie chez un enfant de deux ans. *Arch Franç Pédiat*, 11: 641, 1954.
12. DVORAK, J., KUBOV, A., ZAZVORKA, Z. and HONS, J.: Ein Fall von idiopathischer Hypoalbuminämie mit Aminoacidurie beim Säugling. *Helv Paediat Acta*, 9: 258, 1950.
13. EHRENGUT, W.: Die angeborene idiopathische Ödemkrankheit mit Hypoproteinämie. *Z Kinderheilk*, 74: 141, 1954.
14. WALTER FALK, W. and LARON, Z.: Idiopathic hypoproteinemia. *Ann Paediat (Basel)*, 184: 89, 1955.
15. FANCONI, G.: Über Störungen der Wasser, Kochsalz und Plasmaeiweißhaushalten. *Schweiz Med Wschr*, 76: 791, 1946.
16. FOWLER, J. F. and MILES, J. M.: Rapid protein breakdown in idiopathic hypoproteinaemia. *Strahlentherapie*, Sonderband 38, p. 298, 1958.
17. FREEMAN, T. and MATTEWS, C. M. E.: Analysis of the behaviour of I<sup>131</sup> albumin in the normal subject and nephrotic patient. *Strahlentherapie*, Sonderband 38, p. 283, 1958.
18. GORDON, R. S. JR: The preparation of radioactive polyvinyl-pyrrolidone for medical use. *J Polym Sci*, 31: 191, 1958.
19. GORDON, R. S. JR: Exudative enteropathy. *The Lancet*, 1: 325, 1959.
20. HECHT-JOHANSEN, A.: Hypoproteinaemia. *Acta Path Microbiol Scand*, Suppl. 38: p. 272, 1938.
21. HOLMAN, H. c.s.: Hypoproteinemia antedating intestinal lesions and possibly due to excessive serum-protein loss into the intestine. *Amer J Med*, 27: 963, 1959.
22. HOLTEN, C.: Et tilfaelde af hypoproteinaemi. *Nord Med*, 12: 3059, 1941.
23. HOPKINS, T. R., CONNOR, T. B. and HOWARD, J. E.: Ultrafiltration studies on calcium and phosphorus. *Bull Johns Hopkins Hosp*, 93: 249, 1903.
24. LINGWORTH, R. S. and FINCH, E.: Chronic generalized oedema and hypoproteinaemia of obscure origin. *Arch Dis Child*, 29: 513, 1954.
25. LERSEN, T. O.: Idiopathic hypoproteinemia. *Acta Paediat*, 47: 720, 1958.
26. LUSSEN, E.: Essentielle hypoproteinemie. Thesis, Utrecht. 1957.
27. JONSSON, B. J. and LAGERCRANTZ, V.: Severe hypoproteinemia probably caused by hepatic damage. *Acta Paediat*, 39: 165, 1950.
28. KINSELL, L. W. and MARGEN, S. c.s.: Studies in methionine metabolism III. The fate of intravenously administered S<sup>35</sup>-labelled methionine in normal adult males, in patients with chronic hepatic disease, idiopathic hypoproteinaemia and Cushing syndrome. *J Clin Invest*, 29: 238, 1950.
29. McLEAN, F. C. and HASTINGS, A. B.: Clinical estimation and significance of calcium concentrations in the blood. *Amer J Med Sci*, 189: 601, 1935.
30. MITCHELL, H. H.: Adult growth in man and its nutrient requirements. *Arch Biochem*, 21: 335, 1949.
31. MEYER-BISCH, R.: Über isolierte Störungen des intermediären Salzstoffwechsels und ihre klinische Bedeutung. *Klin Wschr*, 4: 588, 1925.
32. MUSSIO-FOURNIER, J. C., CASTIGLIONI, C. and ANIDO, J. B.: Oedèmes mous, hypoprotéinémie, et anachlorhydrie chez une malade atteinte de goitre exophtalmique. *Rev Neurol (Paris)*, 41: 756, 1934.
33. NONNENBRUCH, W. and KUDLICH, H.: Chronischer Ödemzustand von nephrotischem Typus mit Achropaxie bei chronischer Gastro-enteritis. *Med Klin*, 541, 1931.
34. O'DONOVAN, D. K., McGRATH, J. and GALVIN, C.: Hepatic disease with chronic oedema as sole clinical feature. *Irish J Med Sci*, 6: 519, 1950.
35. PAPPENHEIMER, A. M. and VICTOR, J.: Ceroid pigment in human tissues. *Amer J Path*, 22: 395, 1946.
36. PEARSON, J. D., VEALL, N. and VETTER, H.: A practical method for plasma albumin turnover studies. *Strahlentherapie*, Sonderband 38, p. 290, 1958.
37. SALOMONSEN, L.: Oedema causae ignotae. *Nord Med*, 25: 727, 1945.
38. SCHÖNENBERG, H.: Essentielle Hypoproteinaemie mit Tetanie. *Ann Paediat (Basel)*, 182: 355, 1954.
39. SCHWARTZ, M. and JARNUM, S.: Gastrointestinal protein loss in idiopathic (hypercatabolic) hypoproteinaemia. *The Lancet*, 1: 327, 1959.
40. SCHATZ, M. and THOMSEN, B.: Idiopathic or hypercatabolic hypoproteinaemia. *Brit Med J*, 1: 14, 1957.
41. TEREPKA, A. R.: The ultrafiltrable calcium of human serum II. Variations in disease states and under experimental conditions. *J Clin Invest*, 37: 87, 1958.
42. THORN, G. W. and RENOLD, A. E.: Pathophysiology of edema. *Helv Med Acta*, 23: 334, 1956.

43. ULSTROM, R. A., SMITH, N. J. and HEIM-  
LICH, E. M.: Transient dysproteinemia  
in infants I. *Amer J Dis Child*, 92: 219,  
1956.
44. ULSTROM, R. A., NAKAMURA, K. and HEIM-  
LICH, E. M.: Transient dysproteinemia  
in infants. II. Studies of protein metab-  
olism using amino-acid isotopes. *Amer  
J Dis Child*, 93: 536, 1957.
45. VINK, C. L. J., DOOREN, L. J. and VAN  
GELDEREN, H. H.: to be published.
46. VESIN, P., RENAULT, H. and CATTAN, R.:  
L'hypoprotéinémie idiopathique. Étude  
de son mécanisme par le test au poly-  
vinyl-pyrrolidone- $I^{131}$ . *Bull Soc Méd  
Hôp Paris*, 75: 319, 1959.
47. YOUNG, W. F. and LEVIN, B.: Gastroin-  
testinal protein loss in severe mal-absorption  
complicated by idiopathic (hyperata-  
bolic) hypoproteinaemia. *The Lancet*, II:  
85, 1959.

Submitted Aug. 1, 1960  
Accepted Sept. 13, 1960

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## The Morphological Development of Pulmonary Arteries During the First Years of Life

by H. HERZENBERG and V. ESKELUND

Over the last few years, there has been an increasing interest in the pathology of the pulmonary vessels in congenital and acquired heart disease. The changes in the pulmonary vessels have been shown to play an important part in the symptomatology and prognosis of these diseases. In the last decade, great advances in cardiac diagnosis and surgery have made surgical intervention possible, even during the first years of life. It is very important that clinical data and laboratory findings be correlated with changes in the pulmonary arteries in congenital heart disease, especially during this period of life. In this way, the indications for operation can be more definitely established.

In order to evaluate changes in the pulmonary vessels accurately, an understanding of the normal conditions is a prerequisite. The following is a report on the results of a morphological study concerning the development of pulmonary arteries in children during the first years of life who did not have respiratory or circulatory diseases.

Reports on the development of pulmonary arteries during the first years of life can be

found in the literature, but these are relatively few and the methods vary. For this reason, a direct comparison is difficult. Brenner's nomenclature, from his classic work on the pathology of the vessels of the pulmonary circulation (2) has, as a rule, been the basis for these reports. He divides the intrapulmonary branches of pulmonary arteries into three types: elastic arteries, muscular arteries and arterioles. According to Brenner's definition, the *elastic arteries* are the largest, with a diameter of 1 mm or more, and they are accompanied by cartilaginous bronchi. They have a thin intima which lies directly on a thick, elastic lamina interna. The media is composed of concentric elastic membranes between which are found bands of smooth muscle. The adventitia is made up of rather coarse fibrillar collagen interwoven with fine elastic fibrils. There is no sharp delineation between the elastic arteries and the *muscular arteries* which have an outer diameter varying between 0.1 mm to about 1 mm. These accompany the bronchioli. The thin intima rests on an internal elastic lamina, and the media is composed of circularly arranged smooth muscle between the internal and external lamina. In all of the larger muscular arteries fine elastic fibrils are present in the media. The adventitia resembles that of the elastic arteries.

The muscular arteries merge into the *arterioli*, which are defined as vessels with a diameter of 100  $\mu$  or less. According to



Brenner, the vessel wall is composed of an endothelial tube surrounded by a single elastic membrane which is sometimes interspersed with individual cells of smooth muscle. No elastic externa has been demonstrated by Brenner. It should be pointed out that Brenner's classification is concerned with the conditions found in adults.

Civin & Edwards (3) have shown that the intrapulmonary arteries and arterioles undergo a gradual development from fetal life until adulthood. At birth, the smaller pulmonary arteries resemble corresponding vessels of the systemic circulation. The lumen is narrow and the walls thick. These undergo fundamental changes during the first six months of life. The lumen becomes wider and the vessel wall thinner. Civin & Edwards' work has been verified by Dammann & Ferencz (4, 5), Rosen, Bowden & Uchida (11), as well as Valenzuela, Toriello & Thomas (13). In a special study concerning the development of fetal pulmonary arterioli, O'Neal, Ahlvin, Bauer & Thomas (9) have shown that the amount of smooth musculature in the vessel wall gradually increases during the last part of intrauterine life. They reported that the internal elastic lamina could be demonstrated as early as the latter part of fetal life, even though it was thinner than the external elastic lamina. These authors were thus able to show two elastic membranes in contrast with Brenner's findings, although his material included only adults but no infants or children.

### Materials and Methods

Lung preparations were obtained from autopsy material of 66 patients who died of diseases not primarily affecting the respiratory or circulatory organs. A paraffin block of formalin fixed lung was sectioned (most often several from each patient and from many lobes) and the sections were stained according to a modification of Gomori's elastin staining, developed by V. Eskelund. According to this method the elastin was stained with formalin-fuchsin, after which

the counterstaining (Van Gieson) was done. In this way, the elastic tissue was stained dark blue-violet, and was very distinct.

The vessels are classified according to Brenner's schema, but with one modification. The term "*elastomuscular arteries*" is used when the vessel resembled an intermediate stage between elastic and muscular arteries. These vessels have 2-3 concentric elastic bands in an otherwise muscular media. In addition, the internal and external laminae are relatively thick (Fig. 4). The muscular arteries according to Brenner are divided into *larger muscular arteries* (thus vessels lying near the peripheral branches of the bronchial tree, where respiratory columnar epithelium could be still distinguished) and into smaller muscular arteries. The latter are *precapillary muscular arteries*, which either directly or with arterioli as intermediaries, branch into capillaries. This grouping is made because of the impression that larger and smaller muscular arteries are not always affected by a pathological process to the same extent. Histologically, the precapillary muscular arteries can be seen to branch from a larger muscular artery, lying near a respiratory bronchiole (thus one with respiratory columnar epithelium). Other precapillary muscular arteries may lie surrounded by alveoli, and at a distance from the bronchioli. The precapillary arteries resemble the muscular arteries, and therefore have a definable muscle layer and an internal and external elastica (Fig. 12). Only under pathological conditions is an elastic tissue found in the muscle layer of the media. During the first months of life, the diameter of the lumen for these vessels can be as little as 10  $\mu$ . Arterioli of the type described by Brenner have not been subjected to determination in this material. Various authors define these vessels differently, as it is very difficult to distinguish between arterioli and venu-

The following measurements were made: *diameter of the lumen*, *wall thickness* (consisting of intima and media including external and internal elastic lamina), and *width of adventitia*.

Measurements were taken using a scaled ocular which was adjusted to the user and microscope. As far as possible, vessels with circular lumen were measured (thus vessels which had been cross sectioned). In those cases where such were not present, the smallest diameter of oval-shaped lumens was calculated. In obliquely sectioned vessels, the thinnest width of the wall was measured in order to avoid what might simulate a medial hypertrophy. With regard to the adventitia, which even in cross-sectioned vessels varies in width, an average value was obtained from measurements of the widest and thinnest parts. The values for the adventitia are therefore approximate. At least 10 vessels of larger as well as precapillary muscular artery type were measured. It was not always possible, however, to find so many elastic and elastomuscular arteries in the preparations. On the basis of these measurements, average values were obtained and the variations were recorded. The following ratio was calculated: *Average lumen/average wall thickness (L/M)*

The different ratios at individual ages were graphically compared. Certain cases of chronic diseases and retarded development, where the primary disease did not have its origin from changes in the respiratory and circulatory organs, showed pronounced divergence in the figures, and were therefore not included in the study. It ought to be pointed out that this material, which was fixed in formalin, only partially reflects the conditions *in vivo*, where the vessel calibre greatly varies according to different physiological conditions.

## Results

Each of the four different vessel types has been described according to morphological age development.

**Elastic arteries.** The size of the lumen at birth and some days afterwards is on the average about 300–400  $\mu$ , which

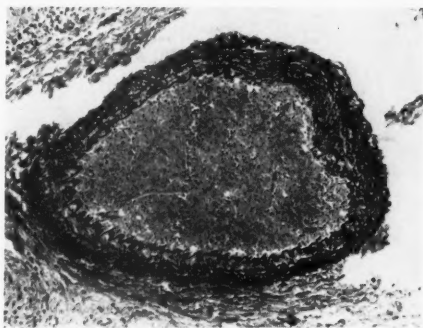


Fig. 1. Elastic pulmonary artery of an infant 2 days old. The internal elastic lamina is thick and prominent and there are numerous elastic fibrils in the media. Part of bronchial cartilage in lower left field. Ratio lumen:wall thickness low (6.0). (This as well as following preparations stained with formalin-fucsin and counterstained with Van Gieson's connective tissue stain;  $\times 105$ .)

corresponds to about one third that found in adults. The internal elastic lamina is thick and wavy, and the same is true of the elastic fibrils which are found in the media (Fig. 1). These contain quite a few smooth muscle cells. Only in certain exceptional cases can collagen fibrils be seen in the outer edge of the media. The external elastic lamina is not quite as thick as the internal, and is sometimes divided. The adventitia is made up of tightly arranged, rather coarse collagen fibrils. Between these are found fine elastic fibrils extending as far as the outer edge, but seen mostly near the external elastic lamina. The adventitial layer is, as a rule, considerably wider than the media. The ratio between the lumen and wall thickness (L/M) is 5–7 (Fig. 2).

By 2–3 weeks of age, the size of the lumen has increased while the wall thickness is to a large extent unchanged. The ratio L/M is therefore larger than earlier

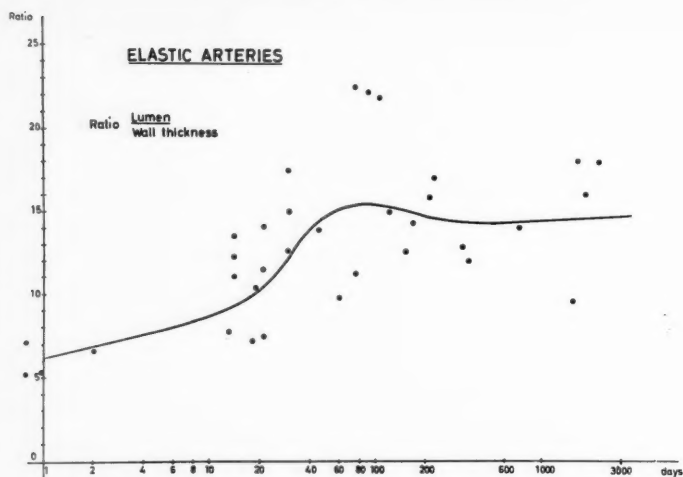


Fig. 2. Elastic arteries. Ratio lumen: wall thickness.

(7-14). The adventitial layer continues to be thicker than the media.

At 1-2 months of age, it is noted that a marked increase in the size of the lumen has occurred at the same time as the width of the wall has decreased. This results in a ratio between lumen and wall more than

double that at birth. The adventitia does not decrease in width as does the media, so that the ratio  $M/A$  decreases.

In the subsequent development, the ratios appear, for the most part, unchanged (Fig. 2). The already fairly sparse smooth muscle cells of the media diminish

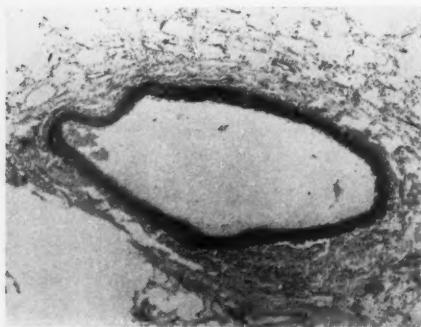


Fig. 3.

Fig. 3. Elastic pulmonary artery of a child 4½ years old. Lumen has increased and wall thickness decreased. Ratio lumen: wall thickness high (15).  $\times 56$ .

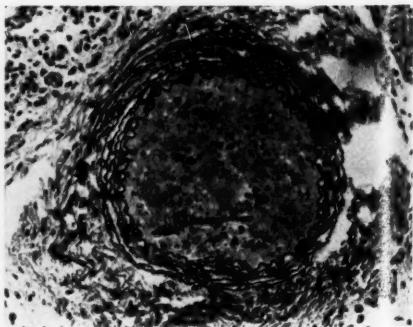


Fig. 4.

Fig. 4. Elasto-muscular pulmonary artery of an infant 2 days old. Media is dominated by smooth musculature but in the middle third there are 2-3 concentric elastic bands. Lumen small, wall thick. Ratio lumen: wall thickness low (7).  $\times 206$ .

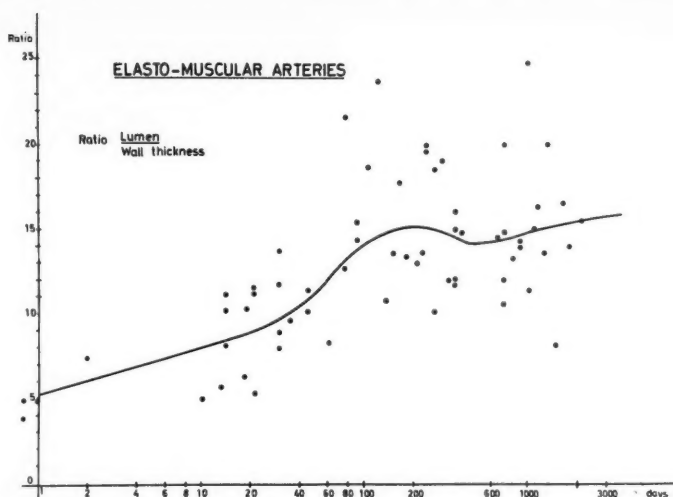


Fig. 5. Elasto-muscular arteries. Ratio lumen:wall thickness

successively. The adventitia decreases likewise in width, but continues to be thicker than the media (Fig. 3).

*Elastomuscular arteries.* As previously described, these arteries are an intermediate form between elastic and muscular arteries.

From birth until about two weeks of age, the average size of the lumen varies between 100 and 200  $\mu$ . The internal elastic lamina is thick and wavy, while the external elastic lamina is thinner and in some places split (Fig. 4). The media is dominated by smooth musculature, but two to three rather coarse continuous elastic bands are found predominantly in the middle third in addition to shorter, thinner, more wavy elastic fibrils. The average width of the wall varies between 200 and 300  $\mu$ . The adventitia is composed of coarse collagen fibrils, usually in rather tight arrangement. Between these are found short and thin elastic fibrils, especially

in the inner third, but sometimes extending to the outer edge. The average width of the adventitia varies between 45 and 75  $\mu$ , and is thus thicker than the media. Because of the comparatively narrow lumen and the thick wall, the ratio L/M is for the most part low, about 4–8 (although in a few cases up to 11) (Fig. 5).

Already from the age of one month, and definitely by 2–3 months, the average size of the lumen has increased (Fig. 6). The media's muscle layer dominates but seems to decrease somewhat, to the advantage of the elastic fibrils. These, as previously mentioned, are coarse and parallel, especially in the middle. The decrease in the wall thickness, together with the enlarged lumen, brings about an L/M ratio (8–22) of more than double the value at birth. The adventitia remains considerably thicker than the media.

During the following months, essentially no changes occur. The adventitia pre-



Fig. 6.

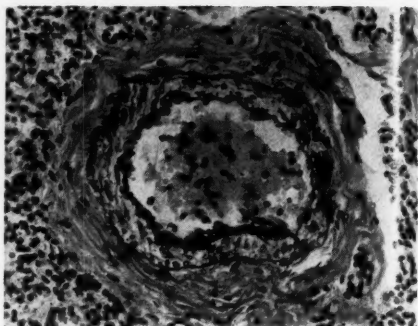


Fig. 7.

Fig. 6. Elasto-muscular pulmonary artery of an infant 3½ months old. A muscular artery is seen branching off to the right. Ratio lumen:wall thickness high (17).  $\times 56$ .

Fig. 7. Larger muscular pulmonary artery of an infant 1 day old. The lumen is narrow and the wall thick. Ratio lumen:wall thickness low (3.5).  $\times 263$ .

serves its greater width compared with the media; the lumen is large and the wall thin.

*Larger muscular arteries.* These arteries lie near those finer branches of the bronchial tree, which still have columnar epithelium. Their media consists of circularly arranged smooth musculature. The transition from elastomuscular arteries is gradual.

At birth and for some days thereafter, the average size of the lumen is 35–80  $\mu$ . The intima's endothelium is evident and, especially in the smaller vessels, the cells often extend out into the lumen. Not infrequently the endothelial cells are loosened. The internal elastic lamina is present in all vessels and, in the larger vessels, is thick and wavy (Fig. 7). The musculature of the media consists of 2–4 cell layers. In the larger vessels thin and short elastic fibrils are found, especially in the middle and out to the adventitia. Collagen fibrils in the media are absent. The external elastic lamina is distinct, but in the larger vessels is not as thick as the

internal elastic lamina, and in some places is slightly interrupted. The adventitia, which is thicker than the media, consists of coarse collagenous fibrils, often rather tightly arranged. In towards the external elastic lamina, a few fine elastic fibrils are seen. Because the wall is thick (average width 9–19  $\mu$ ) and the lumen narrow, the ratio L/M is low, 3–4 (Fig. 8).

At 2–3 weeks of age, the lumen's average size (30–80  $\mu$ ) is for the most part unchanged. In vessels of smaller size the endothelial cells project out into the lumen. The internal and external elastic laminae are seen in all vessels, but the latter continues to be somewhat thinner than the former, and both are sometimes wavy. In the media's musculature, especially of larger but also of middle-sized vessels, thin elastic fibrils are seen. The adventitia is now somewhat more loosely built than at an earlier age. In the central third are found thin elastic fibrils. The ratio between the lumen's size and the width of the wall (L/M) is about 2–6.

By 2–3 months of age, the lumen's

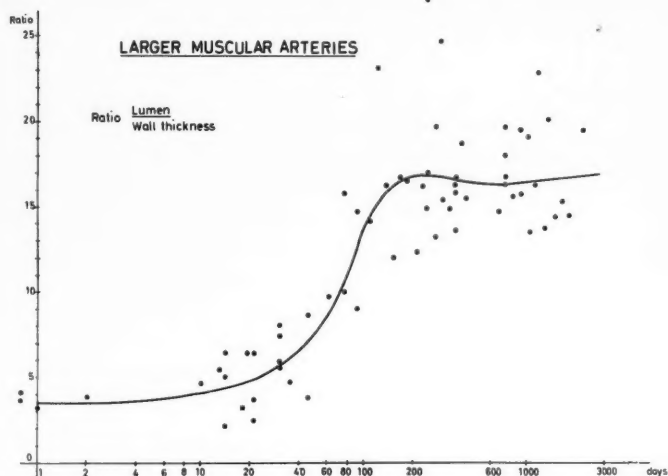


Fig. 8. Larger muscular arteries. Ratio lumen: wall thickness.

average size has generally increased considerably and is now about  $60\text{--}120\ \mu$  (Figs. 9 and 10). The media's musculature has become thinner and consists of 1-3 cell layers with a few thin elastic fibrils. The internal elastic lamina is fairly smooth and is as thick as the external elastic lamina. The adventitia is, as before, thicker than the media. The ratio between

the lumen's size and the wall thickness has, because of an increased lumen and a thinner media, increased to about 9-16, thus 3-4 times more than earlier.

Subsequently, there are essentially no changes, except that the lumen increases somewhat and the wall thickness decreases (Fig. 11). The adventitia remains constant in relation to the media, and is almost

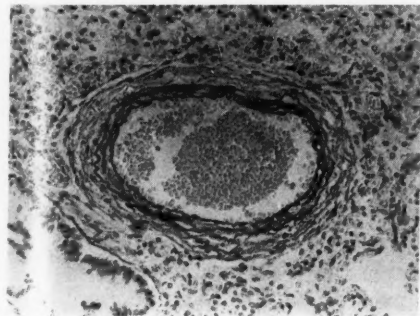


Fig. 9.

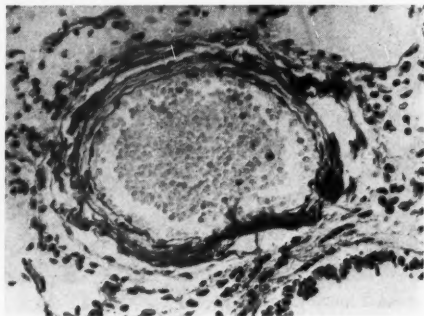


Fig. 10.

Fig. 9. Larger muscular pulmonary artery of an infant 1 month old. Ratio lumen: wall thickness intermediate (10).  $\times 224$ .

Fig. 10. Larger muscular pulmonary artery of an infant  $3\frac{1}{2}$  months old. Ratio lumen: wall thickness (14).  $\times 350$ .



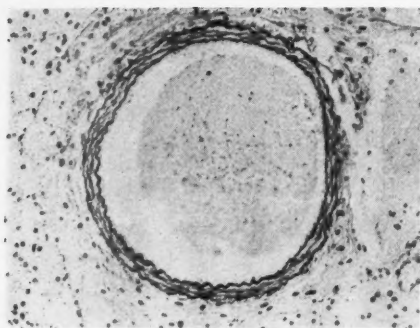


Fig. 11. Larger muscular pulmonary artery of a child 5 years old. Lumen wide, wall thin. Ratio lumen:wall thickness consequently high (15).  $\times 224$ .

twice as thick. The ratio between the size of the lumen and the wall thickness is, as a rule, between 12-27.

*Precapillary muscular arteries.* These vessels constitute, as mentioned previously, the finest muscular vessels before the transition to arterioli and capillaries.

At birth, and for some days afterwards, the lumen's average size is  $9-13 \mu$  (Figs. 12 and 13). The endothelial cells of the in-

tima project into the lumen, and the cells are almost cuboidal. The internal elastic lamina is seen in most of the vessels, but as a rule is thin and interrupted. The media's musculature is composed of 1-2 cell layers. No elastic or collagenous fibrils are seen in the musculature. The external elastic lamina is distinct in all vessels, and is somewhat thicker than the internal elastic lamina. The adventitia is composed of a relatively thick layer of collagen fibrils. No elastic fibrils are present in the adventitia. The wall thickness is, on the average, about  $5-6 \mu$ . The ratio between lumen and wall,  $L/M$ , therefore is thus very low, about 2 (Fig. 14).

By 2-3 weeks of age, the lumen's size increases to about  $10-26 \mu$ . In addition, the elastic tissue has evolved further in the internal elastic lamina, which is now seen in all vessels and is seldom interrupted (Fig. 15). The wall thickness is unchanged, but a few fine elastic fibrils have begun to appear. The external elastic lamina is present in all vessels, but continues to be somewhat thicker than the



Fig. 12.

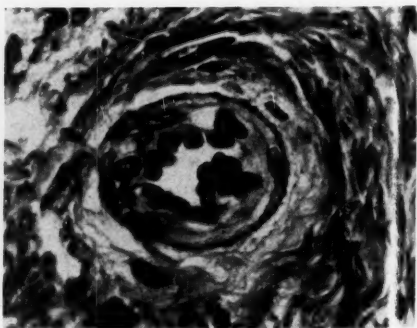


Fig. 13.

Fig. 12. Precapillary muscular pulmonary artery of a stillborn child. The endothelial cells of the intima project into the lumen. Both elastic laminae are seen. Distinct cell nuclei of smooth muscle in the media. Narrow lumen, thick wall. Ratio lumen:wall thickness low (2).  $\times 150$ .

Fig. 13. Precapillary muscular pulmonary artery of an infant 2 days old. Ratio lumen:wall thickness 2.  $\times 1506$ .



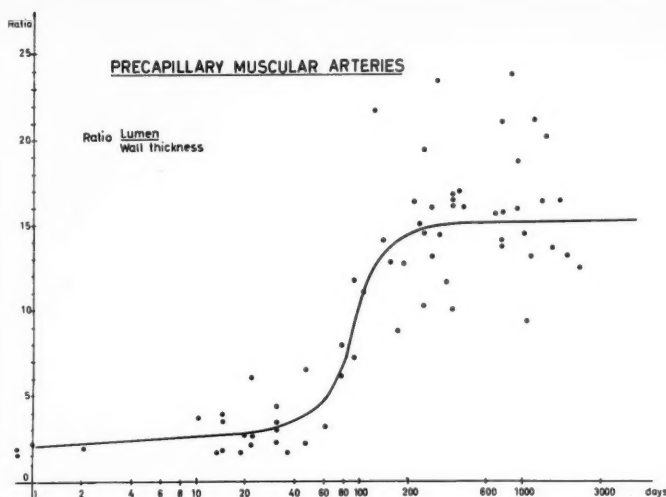


Fig. 14. Precapillary muscular arteries. Ratio lumen:wall thickness.

internal elastic lamina. The adventitia's width is slightly greater than the media's, but is often rather loosely built. A few fine elastic fibrils begin to appear in the adventitia near the border of the media. As no changes occurred in the size of the lumen or the wall thickness, the ratio between these is the same.

At 2½–4 months of age, a big and pro-

nounced change occurs in the size of the lumen (Fig. 16). Its average is now between 19–45  $\mu$ . The intima's endothelial cells are thin and flat, in comparison with their more cuboidal appearance earlier. The internal elastic lamina is as a rule seen, but can be interrupted in a few vessels. The media is composed of only one layer of muscle cells which is occasionally

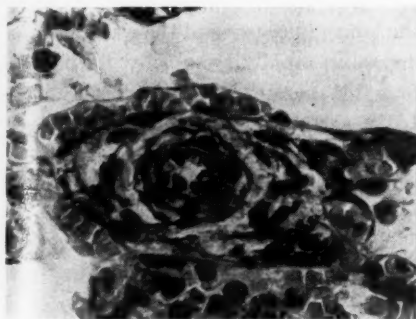


Fig. 15.

Fig. 15. Precapillary muscular pulmonary artery of an infant 1 month old. Ratio lumen:wall thickness (2).  $\times 1103$ .

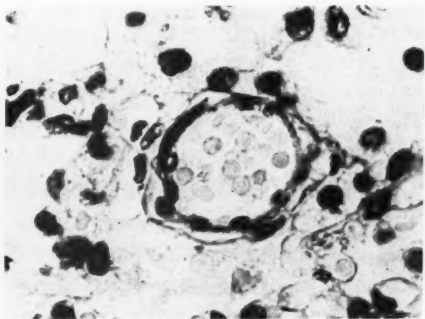


Fig. 16.

Fig. 16. Precapillary muscular pulmonary artery of an infant 3½ months old. Ratio lumen:wall thickness (8).  $\times 1103$ .

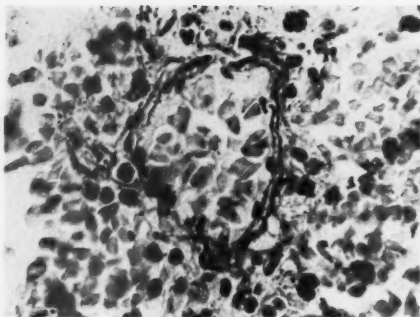


Fig. 17.

Fig. 17. Precapillary muscular pulmonary artery of a child 14 months old. Lumen wide, wall thin. Ratio lumen:wall thickness high (17).  $\times 882$ .

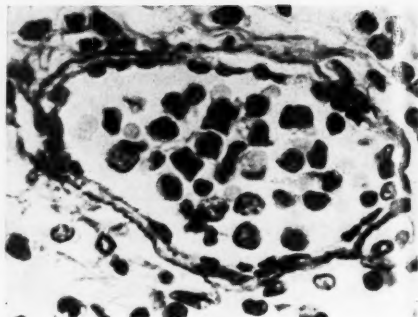


Fig. 18.

Fig. 18. Precapillary muscular pulmonary artery of a child 3 years old. Ratio lumen:wall thickness (13).

interrupted, and has an average thickness if  $2-4 \mu$ . The external elastic lamina is, as previously, distinct in all vessels. It should be pointed out that both the internal and external elastic laminae can be distinguished, even if the musculature is interrupted. The adventitia is often composed of a fairly loosely built layer. The small width of the wall, together with the increasing size of the lumen gives an L/M ratio which is notably increased to about 6-22.

The changes during the subsequent growth are relatively few (Figs. 17 and 18). The lumen, as a rule, is about  $40-50 \mu$ , but variations in both directions can occur. The width of the wall is about  $2-3 \mu$ , and the L/M ratio rarely is under 10. As a rule, the adventitia is greater in width than the media.

### Discussion

At birth and for a few days thereafter, the pulmonary arterial bed (from the elastic arteries to the precapillary muscular arteries) has a characteristic appearance

with narrow luminae and thick vessel walls. The media as well as the adventitia are thick, and the similarity with the systemic arteries is great.

During the first months of life there is a development in that the lumen becomes greater and the vessel wall decreases in width. This change, reflected in the ratio between lumen and wall thickness, takes place slowly at first, is later accelerated, only to become slower again (Fig. 19).

In the larger muscular arteries and the precapillary muscular arteries the size of the lumen increases and the wall thickness decreases rapidly, especially during the first 3-4 months of life (Tables 1 and 2). Later, these changes decelerate, and after 8 months of age they have completely subsided. When comparing these pulmonary vessels and those of a 5 to 6-year-old child, there is no great difference.

Regarding the elastic arteries, the changes in the size of the lumen and the width of the walls occur earlier than in muscular arteries (Tables 1 and 2). The most accelerated development seems to appear as early as 1-2 months of age.

TABLE 1. Average values for lumen, wall thickness and ratio in different ages. (Different pulmonary artery types.)

Artery type	Age		No.	Average values		
	Class boundaries (days)	Class mid-point (days)		Lumen, $\mu$	Wall thickness, $\mu$	Ratio $\Sigma$ lumen/ $\Sigma$ wall thickness
Precapillary muscular arteries	0-29	12	14	14.4	5.5	2.6
	30-119	57	13	22.6	5.6	4.5
	120-299	210	13	41.8	2.9	14.6
	300-749	494	13	45.5	3.6	15.3
	750-	1267	13	54.4	3.6	15.2
Total	—	—	66	—	—	—
Larger muscular arteries	0-29	12	14	55.4	13.3	4.2
	30-119	57	13	83.4	10.0	8.3
	120-299	210	13	136.1	7.5	16.9
	300-749	494	13	127.3	7.8	16.4
	750-	1267	13	151.0	9.0	16.7
Total	—	—	66	—	—	—
Elasto-muscular arteries	0-29	12	14	195.8	24.0	8.2
	30-119	57	13	275.9	25.5	11.8
	120-299	200	11	331.5	21.9	15.1
	300-749	455	12	294.9	21.0	14.1
	750-	1267	13	418.5	27.3	15.1
Total	—	—	63	—	—	—
Elastic arteries	0-17	7	8	459.6	56.0	8.2
	18-44	27	8	600.6	52.3	11.5
	45-119	75	6	738.3	48.2	15.3
	120-359	200	6	533.5	36.6	14.6
	360-	1360	6	709.2	48.3	14.7
Total	—	—	34	—	—	—

The elastomuscular arteries belong to an intermediate group not only from the morphological, but also from the developmental point of view, for their greatest changes occur during 2-3 months of age.

The anatomical changes are reflected in the hemodynamic behavior of the pulmonary circulation during intrauterine, perinatal, neonatal and the early periods of life.

In the fetus, the ductus arteriosus serves as a connection between the right ventricle and the descending aorta. The pressure in the fetal pulmonary artery is somewhat greater than the aortic pressure, so

that only a fraction of the blood which leaves the right ventricle goes to be pulmonary vascular bed (6). On the other hand, the greatest part of the blood is propelled through the ductus arteriosus into the descending aorta and further to the placenta. This shunt is governed by a high vascular resistance in the pulmonary circulation. This high resistance is brought about by the fact that the lungs are collapsed. The pulmonary arterial vessels during fetal life show narrow luminae and thick vessel walls, according to O'Neal *et al.* (9), as well as Larroche, Nodot & Minkowski (8) and thus resemble

TABLE 2. *Smoothed values for ratio lumen: wall thickness at different ages. Change in ratio.<sup>a</sup> (Different pulmonary artery types.)*

Age (days)	Precapillary muscular arteries		Larger muscular arteries		Elasto-muscular arteries		Elastic arteries
	Ratio $\Sigma$ lumen/ $\Sigma$ wall thickness	Change	Ratio $\Sigma$ lumen/ $\Sigma$ wall thickness	Change	Ratio $\Sigma$ lumen/ $\Sigma$ wall thickness	Change	Ratio $\Sigma$ lumen/ $\Sigma$ wall thickness
1	(2.1)		(3.5)		(5.2)		(6.3)
10	2.5	(+ 0.4)	4.1	(+ 0.6)	8.0	(+ 2.8)	8.7
20	2.8	+ 0.3	4.8	+ 0.7	8.9	+ 0.9	10.3
30	3.1	+ 0.3	5.6	+ 0.8	9.7	+ 0.8	12.3
45	3.8	+ 0.7	7.0	+ 1.4	10.8	+ 1.1	14.4
60	4.7	+ 0.9	8.7	+ 1.7	12.0	+ 1.2	15.3
90	8.8	+ 4.1	12.6	+ 3.9	13.7	+ 1.7	15.4
120	12.5	+ 3.7	15.2	+ 2.6	14.6	+ 0.9	15.2
150	13.7	+ 1.2	16.2	+ 1.0	15.0	+ 0.4	15.0
180	14.3	+ 0.6	16.7	+ 0.5	15.1	+ 0.1	14.8
210	14.6	+ 0.3	16.9	+ 0.2	15.1	$\pm 0$	14.6
240	14.7	+ 0.1	16.8	- 0.1	15.1	$\pm 0$	14.5
270	14.8	+ 0.1	16.8	$\pm 0$	15.0	- 0.1	14.4
300	14.9	+ 0.1	16.7	- 0.1	14.9	- 0.1	14.4
330	15.0	+ 0.1	16.7	$\pm 0$	14.7	- 0.2	14.3
360	15.0	$\pm 0$	16.6	- 0.1	14.5	- 0.2	14.3
450	15.1	+ 0.1	16.4	- 0.2	14.2	- 0.3	14.3
540	15.1	$\pm 0$	16.4	$\pm 0$	14.2	$\pm 0$	14.3
720	15.2	+ 0.1	16.4	+ 0.2	14.5	+ 0.3	14.4
1.080	15.2	$\pm 0$	16.6	+ 0.2	14.9	+ 0.4	14.5
1.440	(15.2)	( $\pm 0$ )	(16.7)	(+ 0.1)	(15.2)	(+ 0.3)	(14.6)

<sup>a</sup> Italic figures represent greatest ratio change in different age groups.

the systemic arteries (which are adjusted for a circulation with high resistance).

This characteristic morphology of the larger and precapillary muscular arteries seems to be important in the maintenance of a high intrapulmonary resistance to blood flow in the fetus (3, 9). Another contributing factor is the architecture of the vascular unit of the fetal alveolar duct. Reynolds has shown that in the fetus the terminal arterioles give rise to twisted, coiled capillaries which continue directly into connecting venules (10).

At birth the lungs inflate and normally fill up momentarily with air. With expansion of the lungs the aforementioned coiled vessels become extended and there

is an opening up of a new capillary bed. This capillary plexus can accommodate blood and would account for the increase in the pulmonary arterial blood flow following the first breath. The decrease in the pulmonary vascular resistance after birth would be a result of the extension of the coiled vessels (10). The pressure in the pulmonary artery declines and becomes less than that in the aorta. The flow of blood through the ductus arteriosus reverses its direction and now goes from the aorta to the pulmonary artery, as shown by Adams & Lind (1). That the ductus continues to remain patent and permits a left to right shunt to exist even some time after birth, has been shown

Fig. 19  
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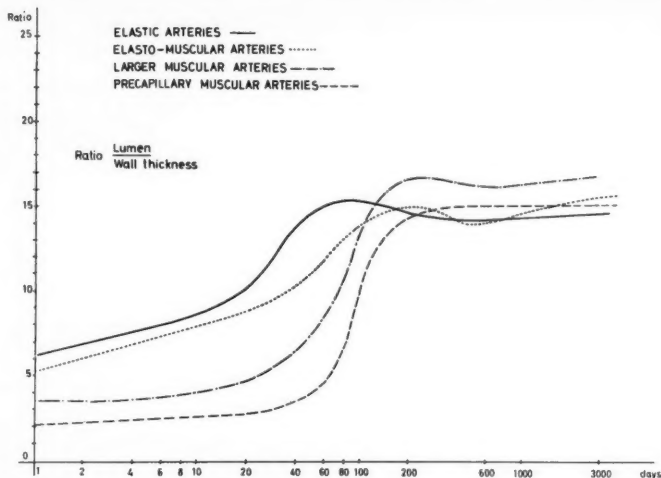


Fig. 19. Comparison of ratios lumen:wall thickness of various types of pulmonary arteries at different ages. The changes in size of vessel lumen and wall thickness occur earlier in elastic arteries than in muscular arteries.

by Rowe & James (12) with cardiac catheterization of newborn mongoloids without congenital heart disease, and by Adams & Lind with catheterization of normal newborn babies. These authors could, with these examinations, demonstrate a left to right shunt through the ductus up to two weeks of age, and an increased flow pressure in the pulmonary artery during the same age period. Rowe & James had 6 cases below 14 days of age with pulmonary artery pressures between 30/10 and 80/50 mm Hg. Adams & Lind demonstrated 6 of 7 cases below 76 hours of age having pulmonary arterial pressures between 31/10 and 50/10 mm Hg. In addition their results show that the pulmonary artery pressure as early as two weeks of age appears to be near the adult value, i.e. ranging between 17/6–30/18 mm Hg, that is 20–25 % of the systemic pressure. From this study, we can demonstrate

that the lumen widens and the thickness of the vessel wall decreases during the first months of life. These changes take place more slowly during the first weeks than they do later. This development seems to be purposeful, for it might contribute to prevent the occurrence of a large left to right shunt through the ductus. Such a large shunt could otherwise bring with it an increased load on the left side of the heart, and subsequently left ventricular failure.

After the first weeks the widening of the lumen and the thinning out of the vessel walls of the pulmonary arterial system occurs more quickly. The wall of the media undergoes a muscular involution. After the fourth month of age, this development again occurs more slowly and after the eighth month of age it is for the most part finished. By then the vessels of the lungs resemble those

characteristic of the adult in whom the vascular bed is adjusted to a circulation with low resistance.

### Summary

The normal pulmonary arterial vascular bed undergoes a morphological development during infancy. The intrapulmonary arteries at birth have narrow luminae and thick vessel walls, thus resembling the structure of systemic arteries. During the first months of life, there is a progressive change, so that the lumen of the intrapulmonary arteries widens and the thickness of the vessel wall decreases.

After 3-4 months of age, this development takes place more slowly, and after 8 months of life has almost subsided. In regard to the different types of intrapulmonary vessels, the elastic arteries (intrapulmonary arteries of greater size) seem to undergo an earlier development than the muscular arteries (smaller type of intrapulmonary arteries). Corresponding to the progressive vascular change during the first months of life, the hemodynamic behaviour of the pulmonary circulation changes from the high-resistance circulation of the fetus to the low-resistance circulation seen in the adult.

### References

1. ADAMS, F. H. and LIND, J.: Physiological studies on the cardiovascular status of normal newborn infants (with special reference to the ductus arteriosus). *Pediatrics*, 19: 431, 1957.
2. BRENNER, O.: Pathology of the vessels of the pulmonary circulation. *Arch Int Med*, 56: 211, 1935.
3. CIVIN, W. H. and EDWARDS, J. E.: Post-natal structural changes in the intrapulmonary arteries and arterioles. *Arch Path*, 51: 192, 1951.
4. DAMMANN, J. F. and FERENCZ, CH.: Clinico-anatomic correlations in "Henry Ford Hospital International Symposium on Cardiovascular Surgery", Saunders, Philadelphia, 1955.
5. DAMMANN, J. F. and FERENCZ, CH.: The significance of the pulmonary vascular bed in congenital heart disease. *Amer Heart J*, 52: 7, 1956.
6. DAWES, G. S.: Advances in Pediatrics. Interscience Publishers, Ltd., London, 1958.
7. EDWARDS, J. E.: Functional pathology of the pulmonary vascular tree in congenital cardiac disease: The Lewis A. Conner Memorial Lecture. *Circulation*, 15: 164, 1957.
8. LARROCHE, J. C., NODOT, A. and MINKOWSKI, A.: Développement des artères et artérioles pulmonaires de la période foetale à la période néonatale. *Biol Neonat*, 1: 37, 1959.
9. O'NEAL, R. M., AHLVIN, R. C., BAUER, W. C. and THOMAS, W. A.: Development of fetal pulmonary arterioles. *Arch Path*, 63: 309, 1957.
10. REYNOLDS, S. R. M.: The fetal and neonatal pulmonary vasculature in the guinea pig in relation to hemodynamic changes at birth. *Amer J Anat*, 98: 97, 1956.
11. ROSEN, L., BOWDEN, D. H. and UCHIDA, I.: Structural changes in pulmonary arteries in first year of life. *Arch Path*, 63: 316, 1957.
12. ROWE, R. D. and JAMES, L. S.: The normal pulmonary arterial pressure during the first year of life. *J Pediat*, 51: 1, 1957.
13. VALENZUELA, C. T., TORIELLO, J. and THOMAS, W. A.: Structural changes in intrapulmonary arteries exposed to systemic pressures from birth. *Arch Path*, 57: 51, 1954.

Received September 2, 1960

Accepted September 16, 1960

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## Respiratory Studies in Children

### II. Functional Residual Capacity in Healthy Children

by F. GEUBELLE and P. DE RUDDER

Engström *et al.* (2) have constructed a diagram representing the value of the lung volumes (residual volume,  $V_R$ ; functional residual capacity,  $V_{FRC}$ ; total lung capacity,  $V_{TLC}$ ),<sup>1</sup> based on the regression of these values on the height measured on 93 children in the 6-14 year age group. The 95 % confidence intervals around the regression lines are also given. When the lung volume of a single subject is being studied, it is possible to estimate the probability that the actual case belongs to a "normal" population.

The influence of training has been studied by Engström *et al.* (2) on 89 double tests with an interval of 10-15 minutes. It has also seemed useful to determine the variability of the lung volumes during a longer interval, i.e. day to day, and to compare the variability with that found in adults.

With the method described by Engström *et al.* (2) three problems have been investigated:

<sup>1</sup> Terms as defined by a group of American clinical and research respiratory physiologists headed by J. R. Pappenheimer (3).

<sup>2</sup> The fixed volume of the system is measured by the simple method described by Engström *et al.* (2). Fifteen determinations of this volume gave a mean value of 7100 ml (range, 7080 to 7150 ml).

1. The accuracy of the method in our hands;

2. The magnitude of the physiological variations of the functional residual capacity ( $V_{FRC}$ ) in healthy children;

3. The comparison between the physiological variation of  $V_{FRC}$  in children and adults.

#### Apparatus

Some differences are to be noted between our apparatus and that of Engström *et al.*: (1) the spirometer capacity is 7000 ml; (2) the catharometer is manufactured by Kipp (Delft, the Netherlands) and the accuracy of the reading is 0.25 % between 0 and 20 %; (3) no membrane pump is used, but a diaphragm on the main spirometer circuit (after the fan type pump and the  $CO_2$  absorber) shunts a part of the expired air through the catharometer; (4) the fixed volume of the system, with the spirometer at its lowest position, is 7100 ml;<sup>2</sup> (5) the helium concentration in the spirometric system before the determination is from 15 to 17 %.

All values are expressed in ml, corrected to body temperature, ambient pressure, saturated with water vapour (BTPS).

#### Material

(1) The volume of the two boxes, whose water capacities at room temperature were



1360 and 2540 ml, was each measured 10 times. The box was connected to the spiographic circuit in the same position as the subject.

(2) To study the physiological variation of  $V_{FRC}$  in children in the sitting position 69 duplicate determinations were performed on 23 apparently healthy children ranging in age from 6 to 15 years old.

(3) To study the physiological variation of the sitting  $V_{FRC}$  in adults and to compare it with that in children, 30 determinations were performed on 10 apparently healthy young adults (students, nurses, etc.).

## Results

### 1. Error of the Method

Assuming the error in the water capacity volume recording to be negligible, the random error of the spirometric method, determined as the average of the random errors for the two boxes, was 22 ml, i.e. about 1%. The error was obtained as the standard deviation of the repeated values. Furthermore, a systematic error exists which seems to make estimates of large volumes about 1% too small. The mean estimated box values were signi-

fically different from the known volume, even though both errors were small.

### 2. Duplicate Determinations

The values of  $V_{FRC}$  are collected in Tables 1-3. Duplicate determinations were made on three occasions for each patient with an interval of some hours to several days. Individual differences between duplicate determinations at an interval of 15 minutes were calculated as the first minus the second determination (Table 4).

The errors of the method (equal to the standard deviation of the differences between duplicate determinations divided by the square root of two) are shown in Tables 5 and 6. The total average is 46 ml or 2.7%. These errors are about twice those obtained with the boxes (22 ml or about 1%).

### 3. Day to Day Variations

(a) The mean values of  $V_{FRC}$  (Table 7) for the first, the second and the third assay in each group do not differ significantly, nor do the individual differences

TABLE 1. Values of  $V_{FRC}$  (functional residual capacity) in 10 healthy adults. Time interval between (1) and (2) is 4 days, between (2) and (3) 6 days.

Height cm	(1)			(2)			(3)		
	$V_{FRC}$ in ml			$V_{FRC}$ in ml			$V_{FRC}$ in ml		
	1st	2nd	Mean	1st	2nd	Mean	1st	2nd	Mean
158	2364	2272	2318	2354	2392	2373	2357	2395	2376
158	1968	1894	1931	1728	1701	1714	1891	1909	900
159	1692	1627	1659	1471	1624	1547	1983	1957	970
160	1773	1751	1762	1363	1518	1444	1719	1686	702
162	1882	1798	1840	2092	2020	2056	2138	2180	159
165	2131	2110	2120	1795	1744	1769	1713	1716	714
176	3719	3529	3624	3439	3524	3483	3059	3080	069
178	3288	3023	3150	3095	2855	2925	2788	2770	779
182	2834	2840	2837	3235	3090	3173	2860	2802	831
183	2891	2876	2884	3208	3106	3157	3129	3091	110

TABLE 2. Values of  $V_{FRC}$  (functional residual capacity) in eleven healthy girls.  $T$  is the time interval between assays, noted in hours (H) or days (D).

Height cm	(1)				(2)				(3)				Age, years
	$V_{FRC}$ in ml			T	$V_{FRC}$ in ml			T	$V_{FRC}$ in ml				
	1st	2nd	Mean		1st	2nd	Mean		1st	2nd	Mean		
104	625	670	650	4H	636	625	630	4D	704	645	675	6.5	
129	698	730	714	4H	769	772	770	5D	686	787	736	11	
145	1607	1612	1609	4H	1445	1447	1446	4D	1610	1700	1665	12	
147	1241	1258	1249	4H	1109	1138	1123	4D	1310	1330	1320	12	
150	1123	1106	1115	4H	1014	1049	1031	4D	1240	1265	1252	11	
153	2110	2077	2093	4D	2098	2098	2098	4H	2490	2345	2417	15	
155	1168	1145	1156	4H	1336	1304	1320	4D	1292	1283	1287	12	
155	1806	1770	1788	4D	1933	1770	1881	4H	1640	1655	1642	13	
158	1364	1414	1389	4D	1257	1242	1249	4H	1347	1307	1327	14	
160	2321	2325	2323	4H	2190	2154	2167	4D	2180	2180	2180	14	
164	1760	1730	1747	4D	1909	1892	1900	4H	2100	1930	2015	15	

TABLE 3. Values of  $V_{FRC}$  (functional residual capacity) in twelve healthy boys. Time interval between (1) and (2) 4 days, between (2) and (3) 6 days.

Height, cm	(1)			(2)			(3)			Age, years
	$V_{FRC}$ in ml			$V_{FRC}$ in ml			$V_{FRC}$ in ml			
	1st	2nd	Mean	1st	2nd	Mean	1st	2nd	Mean	
125	872	851	861	878	849	863	878	870	874	7
131	1178	1097	1137	1017	1087	1052	1040	992	1016	10
136	1094	1005	1049	1000	1080	1040	1062	1014	1038	11
137	1156	1171	1163	1250	1268	1258	1037	1136	1086	9
140	930	931	930	884	884	884	942	910	926	10
140	1214	1233	1223	1134	1118	1126	1215	1205	1210	8
142	1252	1245	1248	1265	1254	1259	1274	1229	1251	13
143	1164	1190	1177	1151	1159	1155	1108	1128	1118	11
149	1574	1539	1556	1460	1505	1482	1558	1516	1537	14
150	1527	1572	1549	1670	1630	1650	1522	1568	1545	14
159	2420	2200	2310	2448	2417	2432	2380	2381	2380	14
171	2496	2445	2470	2439	2421	2430	2467	2521	2494	14

TABLE 4. Individual differences between duplicate determinations of functional residual capacity at three different periods.

M.D. = mean difference, E. = error and S.D. = standard deviation of differences.

	(1)			(2)			(3)		
	$V_{FRC}$ in ml			$V_{FRC}$ in ml			$V_{FRC}$ in ml		
	M.D.	E.	S.D.	M.D.	E.	S.D.	M.D.	E.	S.D.
Adults (10 cases)	+ 82.2 <sup>a</sup>	± 26.9	85.1	+ 22.3	± 41.6	131.5	+ 5.7	± 10.8	34.3
Boys (12 cases)	+ 33.2	± 20.9	75.2	- 6.3	± 11.5	39.7	+ 9.4	± 10.3	35.6
Girls (11 cases)	- 1.3	± 9.5	31.6	+ 24.1	± 20.9	69.6	+ 15.6	± 25.6	84.9

<sup>a</sup> Possibly significant. All other values for M.D. are not significant.

TABLE 5. *Errors of the method in the determination of  $V_{FRC}$  in normal adults (10 cases) boys (12 cases) and girls (11 cases), calculated from duplicate determinations.*

	$V_{FRC}$ (1) ml	$V_{FRC}$ (2) ml	$V_{FRC}$ (3) ml	Average ml
Adults	60	93	24	59
Boys	51	28	25	35
Girls	22	49	60	44
Average	45	57	36	46

TABLE 6. *Errors of the method in the determination of  $V_{FRC}$  in normal adults, boys and girls. Same values as in Table 5 but expressed in percentages of corresponding mean  $V_{FRC}$ .*

	$V_{FRC}$ (1) %	$V_{FRC}$ (2) %	$V_{FRC}$ (3) %	Average %
Adults	2.5	3.9	1.0	2.5
Boys	3.7	2.0	1.8	2.5
Girls	1.5	3.5	4.0	3.0
Average	2.6	3.1	2.3	2.7

when testing the first assay minus the second, the second minus the third and the first minus the third, using only the first determination in the duplicate. The interval times between the assays varied from four hours to ten days.

(b) Since the mean differences tested between assays are not significant, the

physiological variation (i.e., error in one value if physiological variation is reckoned with) may be calculated from the standard deviation of the individual differences (standard deviation divided by the square root of two). Taking only the assays performed at an interval of four days (the largest group according to time interval), the physiological variation during this period is 180 ml for adults, 52 ml for boys and 84 ml for girls. When expressed as a percentage of the mean of the first  $V_{FRC}$  assay in each group, the values are  $\pm 7.5\%$ ,  $\pm 3.7\%$  and  $\pm 5.8\%$  respectively. Thus the physiological variation was between 4 and 8% in these subjects.

### Discussion

In Fig. 1 the three values of  $V_{FRC}$  for each of the boys and girls of our series have been plotted on the diagram of Engström *et al.* (2) to estimate the probability that these children belong to a normal population. Few values lie outside the 95% confidence interval, from which we deduce that these children were normal. However, a more extensive study of Belgian normal material might possibly result in a different regression line (and 95 per cent confidence interval) for  $V_{FRC}$  plotted against height. Andrews *et al.* (1)

TABLE 7. *Mean values for  $V_{FRC}$  in healthy adults, boys and girls. For details, see Tables 1-3.*

	(1) $V_{FRC}$ in ml			(2) $V_{FRC}$ in ml			(3) $V_{FRC}$ in ml		
	M.	E.	S.D.	M.	E.	S.D.	M.	E.	S.D.
Adults	2413	$\pm 213$	672	2364	$\pm 241$	763	2361	$\pm 174$	550
Boys	1389	$\pm 148$	512	1386	$\pm 155$	537	1373	$\pm 156$	540
Girls	1439	$\pm 160$	532	1420	$\pm 159$	528	1501	$\pm 166$	553

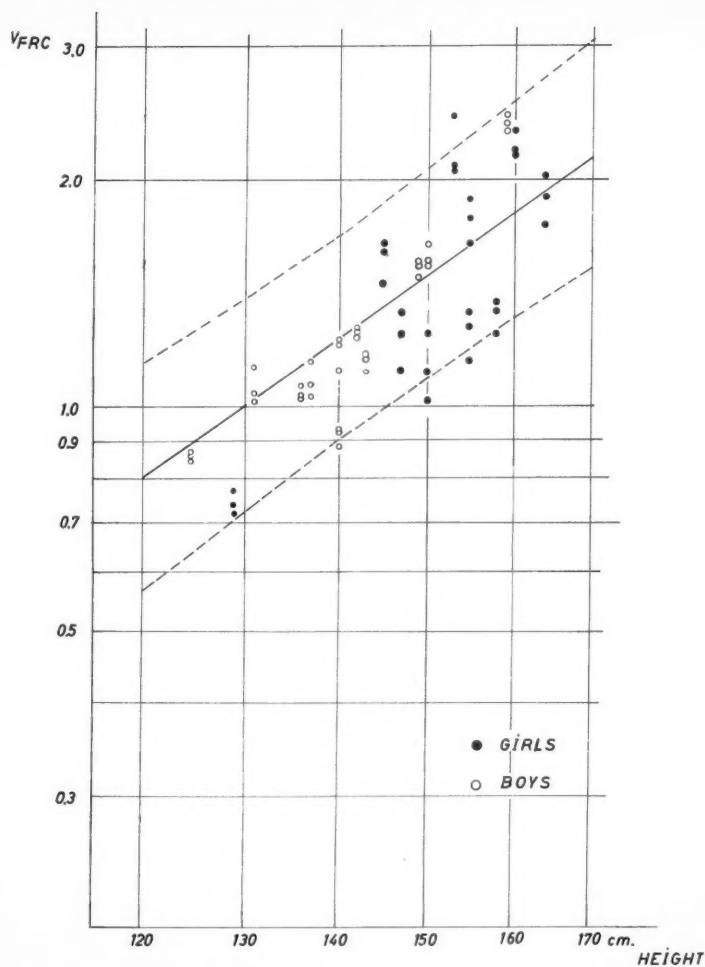


Fig. 1. Values for functional residual capacity ( $V_{FRC}$ ) in healthy boys and girls, plotted against height, together with regression line and standard error ( $\pm 1$  S.D.) of the estimate of the regression equation for the data of Engström, I. *et al.* (1).

have found such small differences on two different materials.

These data show that the functional residual capacity in normal subjects is a relatively stable value with a small day-to-day variation (about 6%). The reason for the variation may be explained by

changes in the chest volume. A day-to-day variation in the position of the ribs, as well as variation in tonicity of the intercostal muscles and the diaphragm would explain the variations of volume of the thorax and, consequently, of the lung.

In a future study the day-to-day varia-

tions of other volumes will be compared with  $V_{FRC}$ . This basic information may well be useful in a day-to-day study of the lung volumes in pathological conditions such as asthma or respiratory muscle paralysis and of the influence of drugs.

### Summary

The functional residual capacity ( $V_{FRC}$ ) was measured in ten normal adults and 23 normal children (11 girls and 12 boys), ranging in age from 6-15 years by a closed

circuit method with helium as the test gas. Three assays, with some hours to several days' interval, have shown a day-to-day variation of about  $\pm 6\%$  in the three groups ( $\pm 7.5\%$  for the adults,  $\pm 3.7\%$  for the boys and  $\pm 5.8\%$  for the girls).

### Acknowledgement

We are very grateful to Dr. A. Solberger, of Stockholm, for the statistical treatment of our results.

### References

1. ANDREWS, J. F. and SIMMONS, D. H.: Lung volumes of normal and asthmatic children. *Pediatrics*, 23: 507, 1959.
2. ENGSTRÖM, I., KARLBERG, P. and KRAEPELIEN, S.: Respiratory studies in children. I.

- Lung volumes in healthy children, 6-14 years of age. *Acta Paediat*, 45: 277, 1956.
3. PAPPENHEIMER, J. R. *et al.*: Standardization of definitions and symbols in respiratory physiology. *Fed Proc*, 9: 602, 1950.

Received August 24, 1960  
Accepted September 16, 1960

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## CASE REPORT

### A Case of Ataxia-Telangiectasia

by JOHN OLOF BONNEVIER

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Louis-Bar described in 1941 (4) the syndrome, later named after her, of slowly progressive cerebellar ataxia and progressive, symmetrical sclerocutaneous telangiectases, based on the case of a 9-year-old boy. In 1958 two cases were published by Centerwall & Miller (3). These authors succeeded in tracing 18 earlier cases, some published and some unpublished, and their series came to consist of 20 cases, 12 girls and 8 boys, from 12 families in Switzerland, the United States of America, England, and Holland. The first Scandianvian case was described in 1959 by Brandt (2), and in 1960 one was reported from Australia (Reye, 5).

The syndrome has been variously named Syndroma Louis-Bar, cephalo-oculo-cutaneous teleangiectases, and, in the American literature, ataxia-telangiectasia. The following features are said to be typical: (1) Hereditary, progressive ataxia commencing in early childhood. The ataxia has been described as being of cerebellar type, sometimes with athetosis. (2) Symmetrically placed telangiectases on the eyeballs, face (sometimes with "butterfly wing" distribution), ears, the exposed areas of back and chest, and hands. (3) Recurrent upper respiratory infections, including sinusitis, bronchitis, and bron-

chiectasis. (4) Indistinct, drawling, monotonous speech. (5) Delayed physical but normal mental development. (6) Dryness of the skin, thin hair with early greying.

#### Case Report

The patient was a girl born on July 14th, 1947. Her parents and 5 siblings were healthy, and the parents are not related. There is no known family history of organic nerve disease or vascular disease. The patient was born at full term and weighed 3300 g, and the delivery was normal. Development during the first year was rather slow, but the child learned to walk by autumn 1948, when she had severe pertussis which lasted for 4 months. She stopped walking, but picked up again, and according to the parents behaved as other children up to the age of 4 years. She then developed slowly progressive difficulties in walking, and jerking and shaking of the arms and hands. She had great difficulty in keeping her balance with the upper part of her body, but learned to ride a bicycle. The symptoms increased, particularly in the arms. When she started school, therefore, she had difficulty in writing. On admission to this paediatric department in autumn 1955 her facial expression was stiff, and she had a slight intention tremor and slight athetotic movements, but the reflexes were normal. She was assumed to have post-pertussis cerebral paresis, and was treated by physiotherapy. Some improvement was thought to take place at times.



Fig. 1. The electroencephalogram on 13/2/1957 showing paroxysmal abnormality at the vertex.

The patient continued slowly to deteriorate, however, and by the age of 8 years she was unable to ride a bicycle.

The child was admitted to another hospi-

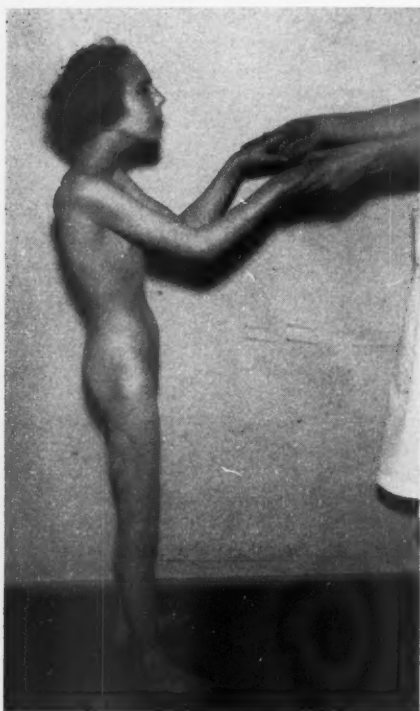


Fig. 2. The patient in February, 1960.

tal in February 1957. She now had a rolling gait, and placed her feet wide apart when walking, but she did not fall. The finger-to-nose and heel-to-knee tests revealed intention tremor. The facies was mask-like, and the conjunctivae were for the first time found to have telangiectases. The electroencephalogram showed a paroxysmal abnormality with a maximum at the vertex and a slight dominance at the left side (Fig. 1). The Wasserman and toxoplasmosis tests were negative. Despite the progressive nature of the illness, the diagnosis was still considered to be cerebral paresis subsequent to pertussis. The girl was treated at an institution for children with cerebral palsy for 6 months during 1957-1958, but deteriorated, and was discharged home. Further deterioration took place, and she could no longer either stand or walk without support. She could dress herself and button up her clothes, but only very slowly owing to severe tremor of the arms and hands. She received school tuition at home a few hours weekly, and learned to read short words and to do simple addition and subtraction. The capillaries of the face became gradually dilated, and later also those of the hands. It is worthy of note that the patient, according to her mother, had never had a cold, and that she had been less susceptible to infection than her siblings.

In February 1960 the patient was re-admitted to the paediatric ward at Västerås. She was small and, as can be seen from Fig. 2, thin and round-shouldered, with the head

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Fig. 3. Telangiectases on the left eyeball and upper and lower lid.



Fig. 4. Telangiectases on the right hand.

projecting forwards. The arms, legs, hands, and feet were thin and atrophic, with thin, dry, cyanotic skin. Telangiectases were present symmetrically on the sclerae, eyelids, chin, neck, occiput, and dorsa of the hands (Figs. 3 and 4). She seemed "absent-minded", but answered questions satisfactorily.

Uncontrolled, sudden, jerky movements of the arms and head took place occasionally, especially in connexion with voluntary movements and when the patient was concentrating. The facies was almost entirely expressionless, but on request she was able to produce a smile or similar movements. There was loss of muscle power, and the musculature showed symmetrical atrophy. The perception of light-touch and cutaneous pain was normal. There was marked ataxia with violent intention tremor and concomitant tremor movements of the other arm, head, and trunk. The child could not walk, even with support, and could not stand unaided. The tendon reflexes were present but weak. Babinski's sign was negative. The ocular fundi were normal. The visual acuity was 0.2 on both sides (Monoyer's scale). Skyscopy gave the following results: right

eye, 0 - +1 D; left eye, 0 - +0.5 D. The ophthalmologist regarded the impairment of vision as being of central origin. It was not possible to carry out a full vestibular investigation; the caloric reactions were normal, however. Hearing was normal, and so was the electroencephalogram. X-ray of the skull showed a rather small skull-cap. Lumbar puncture was not done. Analysis of blood and urine gave normal results. The intelligence quotient measured by the Terman-Merill method was 43, although this seemed low with regard to the child's development. This figure and the general impression gained of the patient were probably due to her motoric disorder and impairment of vision. The patient was discharged home without further treatment.

### Discussion

The diagnosis of ataxia-telangiectasia in this case is based on the progressive cerebellar ataxia, the patient's appearance, and the typically placed telangiectases.

As far as can be determined, the

parents are not blood relations, and there is no family history of organic nerve disease or of skin or vascular changes similar to those seen in the condition under discussion. The 20 cases collected by Centerwall & Miller in 1958 originated from 12 families, which would suggest the presence of a hereditary factor, although in several instances this was not confirmed. The absence of a family history in our case is therefore no bar to the diagnosis.

It was first thought that the child had cerebral palsy following pertussis. The patient has since been followed up over a period of several years, however, and the nature of the illness has become clearer. During the first few years the disease progressed so slowly that it was compensated by the physical and mental development of the child. The patient's condition seemed to be stationary. During recent years the picture of progressive ataxia has become more and more obvious.

The skin lesions consist of telangiectases symmetrically localized to those parts of the body that are exposed to sunlight. This may perhaps be interpreted as an expression of photosensitization. The syndrome might therefore be related to other states in which photosensitization and lesions of the central nervous system occur together. In Hartnup's disease a pellagra-like rash develops on areas of the skin directly exposed to sunlight, and there is reversible cerebellar ataxia. These patients have in addition constant aminoaciduria. Our patient has not been examined for this feature, and aminoaciduria was not present in any of the children examined by Centerwall & Miller.

The relationship between the telangiectases and the neurological findings may

be considered to be established. The central nervous system was indeed normal on macroscopical examination in 2 necropsies (1, 3), but histological examination revealed thickening of the meninges and widening of the meningeal vessels. Similar vessels were also reported in the parietal and frontal lobes, nucleus dentatus, and the olives. There was a greatly reduced number of Purkinje cells, and there were signs of diffuse, primary, progressive degeneration of the cerebellar cortex. Air encephalography had shown dilatation of the 4th ventricle, indicating cerebellar atrophy. Both patients had died of pulmonary infection, and the necropsies revealed inflammatory changes of the lungs but no vascular lesions. The relationship between the cephalo-oculo-cutaneous telangiectases and the repeated infections is therefore not explained.

The mental development of these patients is usually within normal limits. Owing to the expressionless facies, however, it commonly appears to be delayed, and the impairment of motor function is accompanied by a corresponding inability successfully to perform the standard tests. The intelligence quotient of 43 obtained by the Terman-Merill method in our patient was, as the tester pointed out, undoubtedly too low. Having regard to the child's ability to read and do simple sums, and to her behaviour, it is probable that the true value is a good deal higher, but it is unlikely that her mental development is within normal limits. Boder & Sedgewick report intelligence quotients of 91-107 for 5 children tested at the age of 9 years, but later tests on the same children resulted in values of 71-86.

The susceptibility to infection and the

repeated upper respiratory tract infections have been regarded as an important part of the syndrome. The infections have commenced at varying ages, but always between 2 and 8 years. In our case the susceptibility to infection would seem to have been unusually low, despite the fact that the child has 5 brothers and sisters and would therefore be particularly exposed to infections. Nothing is mentioned in Louis-Bar's original article or in that of Wells & Shy (6), who describe 2 cases, about prolonged infections, although all 3 patients were about 9½ years old. Susceptibility to infection would not therefore seem to be an essential feature of the syndrome.

Ataxia-telangiectasia may probably be regarded as an hereditary ataxia, since the course of the illness does not differ essentially from that of others of the same category. The syndrome should be grouped among the angiomas with changes in the central nervous system. These include

Sturge-Weber's syndrome, in which there are angiomas of the cortex and face, and Osler's hereditary telangiectasia which may involve the brain, skin, eyes, and lungs. In the latter state there is no ataxia, however. Finally, von Hippel-Lindau's disease, commencing during childhood, may be mentioned, in which there is progressive angiomatosis of the retina and cerebellum, progressive impairment of vision, and ataxia.

The clinical picture in these states is not identical to that in ataxia-telangiectasia, which should therefore be distinguished from other angiomas.

### Summary

Report of a 13-year-old girl with progressive ataxia starting at the age of 4 years, and later also oculo-cutaneous telangiectases. Points of divergence from the findings in ataxia-telangiectasia are discussed.

### References

1. BODER, E. and SEDGWICK, R. P.: Ataxia-telangiectasia. *Pediatrics*, 21: 523-526, 1958.
2. BRANDT, S.: Cephalo-oculo-cutane telangiectasier (Louis-Bar's syndrom). *Nord Med*, 62: 1519-1521, 1959.
3. CENTERWALL, W. R. and MILLER, M. M.: Ataxia, telangiectasia and sinopulmonary infections. *Amer J Dis Child*, 95: 385-396, 1958.
4. LOUIS-BAR: Sur un syndrome progressif comprenant des télangiectasies capillaires cutanées et conjunctivales symétriques, à disposition naevioide et des troubles cérébelleux. *Confin Neurol*, 4: 32-42, 1941.
5. REYE, C.: Ataxia-telangiectasia. *Amer J Dis Child*, 99: 238-241, 1960.
6. WELLS, C. E. and SHY, G. M.: Progressive familial choreoathetosis with cutaneous telangiectasia. *J Neurol Neurosurg Psychiat*, 20: 98-104, 1957.

Submitted Aug. 4, 1960

Accepted Oct. 26, 1960

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CASE REPORT

## Premature Synostosis in the Sternum (Silverman's Disease)

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Of the various congenital anomalies which affect the sternum, the most common is funnel chest. Premature synostosis is a much rarer sternal anomaly and is characterized by premature obliteration of the sutures between the sternal segments. This results in the characteristic deformity which is similar in appearance to pigeon breast.

The sternum is made up of the manubrium, four mesosternal segments, and the xiphoid process. Ossification of the centres in the manubrium and in the three proximal segments of the mesosternum usually begins during the last 3 or 4 months of foetal life, whereas the distal segment of the mesosternum and the xiphoid process generally do not ossify until after birth (2). Fusion of the different segments, which takes place from below upwards (Fig. 1.), is completed between the 16th and 25th years of age, whereas the manubriosternal synchondrosis remains unfused until after the age of 30 in 90 % of people (1).

### Case Report

The patient, a 7-year-old boy, was the third of four children. There was no family

history of hereditary diseases or malformations, particularly no history of chest deformities. His birth history was normal. He was admitted for the first time, at the age of 16 days, with a referred diagnosis of heart disease and was found to have Fallot's tetralogy combined with a patent ductus arteriosus. The patient was subsequently admitted seven times because of his heart disease, most recently at the age of 7 in October 1959. Despite this he got on surprisingly well, developing almost normally for his age, although at times he had moderate dyspnoea and cyanosis of his lips. X-ray investigation, as early as the age of 16 days, revealed incipient synostosis of the sternum. Repeated X-ray films at the age of 6 months showed more marked changes (Fig. 2), and at 7 years showed fairly marked deformity, consisting of pronounced forward angulation of the sternum (Fig. 3). Clinically, the chest was deformed, showing a midline bulge of the sternum (Fig. 4).

### Discussion

Only ten cases of premature sternal synostosis have been reported previously, most of them by Currarino & Silverman (3). None was demonstrated in newborn infants, so that it cannot be stated definitely whether in some of the reported cases the sternum has formed from one ossif-

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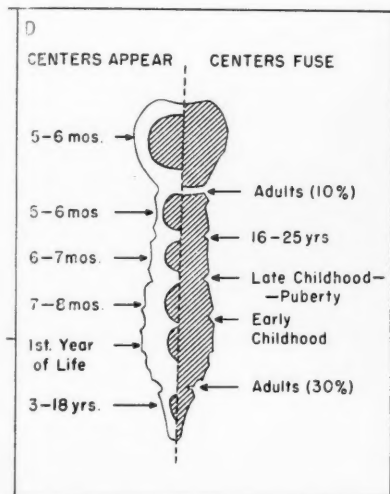


Fig. 1. The normal development of the sternum and ossification of the centres (after Silverman).



Fig. 2. Lateral roentgenogram of the chest of the patient when 6 months old. Note the forward angulation of the sternum and narrow sutures.

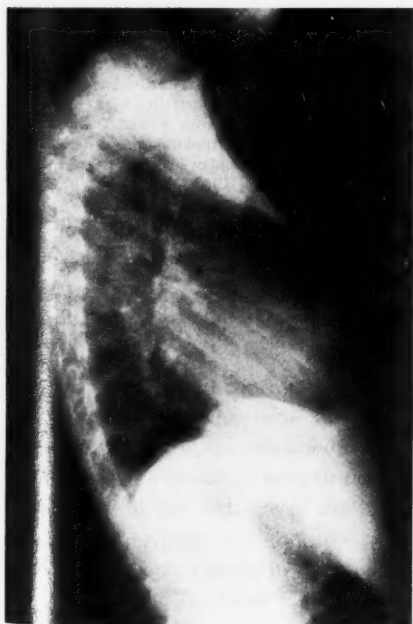


Fig. 3. The same patient at the age of 7 years. Complete obliteration of the sutures with pronounced deformity of the sternum.

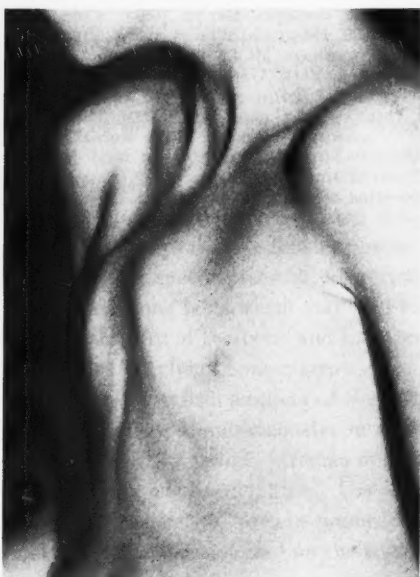


Fig. 4. Photograph of the boy, aged 7 years, with pigeon breast.

tion centre or whether the centres have fused after birth. Silverman mentions a patient, aged 3 days, who displayed slight forward angulation of the sternum with narrow sternal sutures. The youngest case on record was 1 month old (4). In the present case, X-ray examination 16 days after birth showed that the sutures were narrow although segmentation was still present. The characteristic X-ray finding in all previous cases as well as in the present one is the fairly marked forward angulation of the sternum. There is no pathological evidence that the ribs or the diaphragm play a causal rôle in the premature fusion of the sternum.

It is remarkable that six of the ten previously reported cases have had congenital cardiovascular malformation, usu-

ally in the form of a shunt and signs of pulmonary stenosis. Since the present patient also has congenital heart disease, it seems reasonable to think of a common disturbance in embryologic development. Premature synostosis of the sternum should be looked for in patients with congenital heart disease particularly those with intracardiac shunts or pulmonic stenosis. It may be more common than the literature would suggest.

### Summary

A case of a rare sternal anomaly, premature synostosis, is reported, characterised by premature obliteration of the sutures between the sternal segments. The clinical and roentgenologic findings are discussed.

### References

1. ASHLEY, G. T.: The morphological and pathological significance of synostosis at the manubrio-sternal joint. *Thorax*, 9: 159, 1954.
2. CUNNINGHAM, D. J.: Textbook of Anatomy. New York, Oxford University Press. 9th ed., 1951.
3. CURRARINO, G. and SILVERMAN, F. N.: Premature obliteration of the sternal sutures

and pigeon-breast deformity. *Radiology*, 70: 532, 1958.

4. MONNET, P., GRAVIER, J., GAUTHIER, J. and VERNEY, R.: Association d'une transposition vasculaire partielle (dextroposition aortique) avec retour veineux anormal et d'une malformation thoracique par ossification prématurée du sternum. *Pédiatrie*, 11: 95, 1956.

Received June 15, 1960  
Accepted Sept. 13, 1960

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## CASE REPORT

### Subperiosteal Haemorrhage in Haemophilia A and B

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In classic haemophilia (haemophilia A) as well as in Christmas disease (haemophilia B) severe skeletal anomalies may occur owing to repeated haemorrhages.

These anomalies are usually caused by repeated haemorrhages into the joints; the joints most frequently affected are the knees. Due to repeated haemarthrosis the synovia becomes hypertrophic and more vascularized; then the cartilage becomes affected causing irregularity of its surface.

Changes in the subchondral bone also take place, initially in the form of sclerosis and cysts. If the intra-articular haemorrhages occur frequently, the patient does not rest sufficiently, and if no orthopaedic measures are taken nor measures for obtaining amelioration of the blood anomalies, then the deviations in the joint cartilage and in the subchondral bone increase steadily (compare, *inter alia*, Jordan (3), Middlemiss (4)). This will result in severe malformations of the joints with impaired mobility and contractures.

Haemorrhages in the bone may also occur; this may be in the subchondral part of the bone, in connection with degenerative changes in the cartilage of the joint. But the haemorrhages can also take place in the growing epiphysis or in the diaphysis of a long bone. The latter may result in

a picture which, for instance, in the hip-joint resembles very much Perthes' disease.

At first the bleedings in the shaft may give the impression of a bone cyst in which the necrotic bone is completely resorbed. But in this necrotic area calcification may also occur; so that in the shaft a markedly calcified zone in the midst of an ischaemic zone is visible.

And finally a subperiosteal bleeding may occur also; this is a more rare occurrence. Occasionally a periosteal reaction will be found in the neighbourhood of an affected joint as a symptom of reactive hyperaemia. In the subperiosteal bleeding calcification may occur, but later on resorption of the newly formed bone takes place, so that the condition again becomes normal.

However, occasionally a repeated subperiosteal and capsular bleeding may occur. In this way a severe pressure is exerted on the underlying bone which may lead to pressure atrophy of the bone and later on also of the underlying bone-marrow.

Usually a large firm swelling of the soft parts is present simultaneously; in such cases we speak of a pseudo-sarcoma or of a pseudo-tumor in haemophilia. The development of such a pseudo-tumor depends on its localization and on the treat-





Fig. 1. Patient R. Date: 5/3/59.

ment. In principle there is no difference between the bone anomalies which may occur in haemophilia A and in haemophilia B, though cases of bone anomalies in haemophilia B are still relatively few.

We were able to observe such a pseudo-tumor in two patients, one suffering from haemophilia A, and in one with haemophilia B.

**CASE 1.** Patient R., a boy aged seven years and a half, is known to suffer from haemophilia A. Since he was one year old, he has been admitted repeatedly to our clinic, especially in the first years of life on account of haemorrhages of the skin, of frenulum and gums, but since his sixth year also because of repeated joint haemorrhages.

When six years and four months old he was admitted again, this time on account of an extensive bleeding in the area of the right hip-joint. On admission the boy was very pale and showed a severe swelling from 5 cm below the right crista iliaca to 15 cm above the right knee. The mobility in the right hip was greatly restricted. The haemoglobin content of the blood was 6 g%. In the next three weeks the boy received transfusions of 2750 cc blood and 2250 cc plasma; then the haematoma had been resorbed for the greater

part and the hip could be moved slightly; however, a paralysis of the sciatic nerve developed. On account of an incipient contraction a suspension-stretch bandage was applied and one month after admission electrotherapy of the muscles of the lower leg was started. However, in the long run a more adequate revalidation therapy was necessary and therefore the patient was transferred to the "De Hoogstraat" revalidation centre in Leersum. When the boy had been there for several months, he developed a large haematoma of the right heel, which within some days reached alarming proportions. Now the boy was transferred again to our Children's Clinic.

On admission the patient did not give the impression of being seriously ill; his feeling was excellent. Temperature 39.1°C. No abnormalities of eyes, ears, nose, mouth and throat, neck, breast or abdomen. A flexion contraction of the right hip and right knee was present. The right heel was enormously swollen and as big as the fist of an adult man (Fig. 1). The skin above the swelling was red, smooth and stretched with increased venous pattern. There was a slight talipes equinus. The colour of the forefoot and of the toes was normal. The toes could move normally. The X-rays showed a marked atrophy of the bone; about half of the right calcaneus had disappeared (Fig. 2).

For the diagnosis we had to consider the possibility of haemorrhage, a malignant tumour or osteolysis. BRUMMELKAMP (1) recently described the picture of pseudosarcoma in haemophilia and discussed the difficulties of the differentiation in these conditions with malignant tumours. VAN DE WEYER (5) at a meeting of the Dutch Orthopaedic Society communicated his experiences with two patients with osteolysis ("dite essentielle"), a mysterious morbid picture in which, without any obvious reason, a part of the skeleton totally disappears.

A diagnosis of osteolysis in a patient with haemophilia A was made. The following therapeutical points had to be considered:

- (a) prevention of decubitus of the highly stretched skin.
- (b) promotion of the resorption of the enormous haemorrhage, largely of old blood, by means of frequent transfusions of fresh blood or of fresh plasma (citratd or heparinized plasma) over a long period.
- (c) prevention of new contractions.
- (d) the already existing contractions had to be restored to a normal condition.

With reference to (a) the boy had to change his position in bed regularly. In order to promote the resorption of the haemorrhage the boy received transfusions of 150 cc of heparinized plasma immediately after his admission, next day a transfusion of 500 cc of blood and from this day on a daily transfusion of fresh citrated plasma (Van Creveld & Mochtar (2). With regard to (c) and (d) the boy was encouraged to move all joints of the right leg actively.

The further course was favourable. Some days after admission the pain in the right foot abated considerably; on the third day the temperature had dropped to

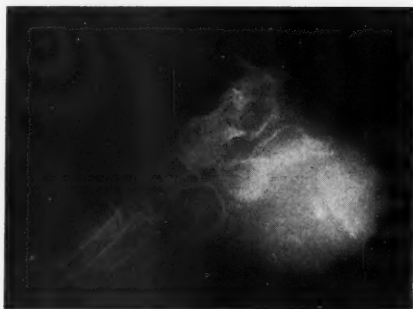


Fig. 2. Patient R. Date: 5/3/59.

normal values and the swelling gradually became smaller. After three weeks the skin over the heel was wrinkled, and the daily transfusions were discontinued.

After six weeks there was a considerable decrease of the swelling; at that moment a distinct pes equino-varus as well as the persisting and still existing paresis of the nervus ischiadicus was present. The poor position of the right foot was fixated and the roentgenogram showed a recalcification of the os calcaneum (Fig. 3). Then the position of the foot was ameliorated by cautious manual redressment under protection of a plasma infusion, and the foot was placed in plaster. Every two weeks this redressment was repeated, always under protection of a plasma infusion.

After three months a satisfactory position of the foot was obtained. No more haemorrhages occurred. The plaster-cast was replaced by a walking cast and the patient was mobilized.

About five months after the admission, roentgenograms revealed a recalcification of the calcaneus, though its form is somewhat different from the previous ones (Fig. 4); the skeleton of the foot still shows some general atrophy. The contractures

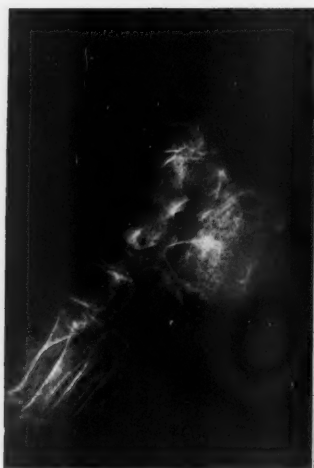


Fig. 3. Patient R. Date: 15/4/59.



Fig. 4. Patient R. Date: 12/8/59.

of hip and knee have disappeared. The neurological deviations have greatly improved; there is still a peroneal paresis, and on account of this the foot shows an inclination to become a club-foot. For this reason the patient again received a walking cast, which later on was replaced by a leather support.

CASE 2. Our second observation is that of patient W., a boy with haemophilia B, now nine years old, and only child of healthy parents. There are no haemorrhagic diatheses in the family. An intracranial haemorrhage occurred at birth.

During the first four years he often suffered from fainting fits, blue spots and nose-bleedings. Then no more haemorrhages occurred till at the age of seven years he fell with his right knee on a fragment of glass. The wound became infected and surgical intervention was followed by a haemorrhage in the right knee. The patient received a plaster cast for both legs and was admitted into the Children's Clinic for the first time. The haemarthrosis was reduced by means of ice-bags, transfusions of blood and plasma and continuous nursing in plastersplints.

The restricted extension of the right knee was completely restored to normal by means of a plaster stretch bandage. After a stay of about two months in the Children's Clinic the patient was discharged.

Some months later, repeated haemorrhages occurred, *inter alia* in the right knee, and a haemorrhage in the right heel developed. The roentgenogram of the foot revealed that the greater part of the calcaneus had disappeared. In consultation with the paediatrician (Dr. Wijffels) the boy, now aged eight years, was readmitted to the Children's Clinic.

The principal abnormality was localised in the right heel. Laterally and at the posterior of the heel a large and red blue swelling, the size of a grape-fruit, was present. The skin of the heel was smooth and stretched. The roentgenogram of the calcaneus gave the picture as seen in Fig. 5. A talipes was present. The foot was placed in a suspension bandage in order to free the heel from any pressure. The patient was encouraged to make active movements with his feet. Then an intermittent infusion of fresh citrated plasma or lyophilized heparinized plasma was started and continued for 14 days. A week after the beginning of this

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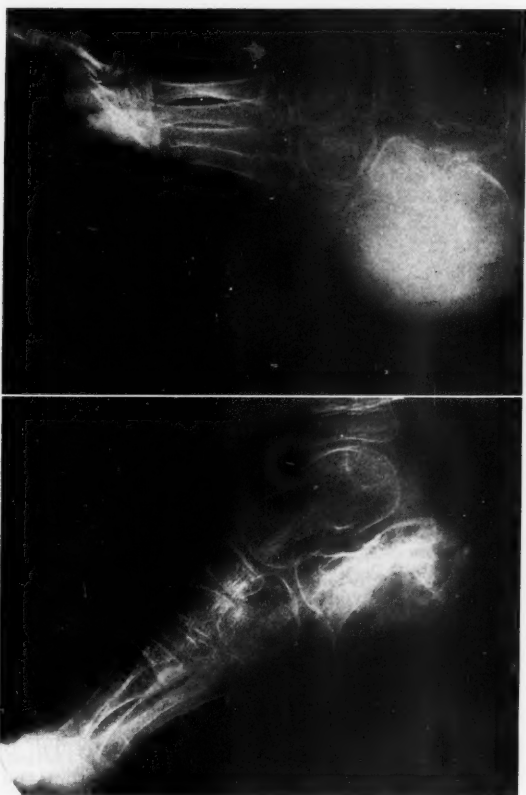


Fig. 5. Patient W. Date: 24/11/59.

Fig. 6. Patient W. Date: 13/2/60.

treatment the skin was less smooth and less stretched. The sensitivity was undisturbed and the toes could be moved in a normal way.

A month after his admission exercises under supervision of a physiotherapist were started. The swelling and discoloration gradually diminished and the regular check by means of roentgenograms showed an increasing calcification in the osteolytic area (Figs 6 and 7).

Three months after the admission the boy started walking between walking bars with

no weight-bearing on his right foot. Two weeks later a haemorrhage in the right knee occurred which soon disappeared after several transfusions of plasma. Still some weeks later another haematoma occurred. Now the walking exercises were discontinued for some weeks, and about 7 months after admission these exercises were cautiously started first for some weeks under supervision in walking bars and after that with two crutches. In the meantime the regular roentgenologic control of the heel had shown an important improvement of the ossification (Fig. 8).



Fig. 7. Patient W. Date: 12/5/60.



Fig. 8. Patient W. Date: 9/9/60.

### Discussion

In our opinion the combined treatment with transfusions of blood and plasma and orthopaedic measures have prevented a further progression, as has been observed in other cases.

Stimulating the resorption of the haematoma undoubtedly has promoted the deposition of the new bone. This was localised largely within the original limits of the affected part of the skeleton. Finally the very cautious mobilization has contributed to the fact that the haemorrhage did not recur immediately.

### Summary

Description of two cases of extensive subperiosteal haematoma of the heel in a patient with haemophilia A and in a patient with haemophilia B. These haematoma gave rise to extensive destruction of the calcaneus. Discussion of the means by which recovery was obtained.

We are grateful to Dr. H. A. E. Fermin and his staff from the Roentgenological Department of the Binnen Gasthuis for the preparation of the X-ray films.

### References

1. BRUMMELKAMP, W. H.: Pseudo-tumeur en cas d'hémophilie. *Arch Chir Neerl*, 10: 263, 1958.
2. CREVELD, S. VAN and MOCHTAR, I. A.: Management of Hemophilia. Hemophilia and Other Hemorrhagic States. University of North Carolina Press, Chapel Hill, N.C., U.S.A. p. 27. 1959.
3. JORDAN, H. H.: Haemophilic Arthropathies. Charles C. Thomas, Springfield, Ill. U.S.A., 1958.
4. MIDDLEMISS, J. H.: Haemophilia and Christmas disease. *Clin Radiol*, 11: 40, 1960.
5. WEYER, F. A. VAN DE: Osteolyse "di essentielle". *Ned T Geneesk*, 104: 545, 1960.

Submitted Sept. 18, 1960

Accepted Oct. 5, 1960

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## CASE REPORT

# Renal Tubular Defects in Fibrous Dysplasia of the Bones

## Report of Two Cases

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The purpose of the present paper is to report the occurrence of renal tubular defects in two cases of fibrous dysplasia of the bones (f.d.b.).

CASE 1. G. B., a girl, was born in 1946. Her parents are unrelated and healthy. Three brothers are also well. No other case of congenital anomalies in the family is known. Pregnancy and delivery were normal. Birth weight 3500 g, height 52 cm. The neonatal period and psychomotor development were normal. She was nursed for two months, then bottle-fed on prescribed formula. Cod liver oil was given from four weeks of age through the first year, and later every winter in appropriate prophylactic doses. From the age of five years she started to complain of pains in the legs and she tired easily. Accelerated growth and development of the breasts was noted, and pubic and axillary hair had developed. Her body proportions were more mature than expected for her age. Her first menstruation occurred at nine years of age. At the age of seven she was admitted to the University Pediatric Clinic, Rikshospitalet, for the first time, because of the leg pains and premature sexual development. She was then tall, height 134 cm (97.5 percentile for age), weight 30 kg (50 percentile for height) and had a broad and heavy trunk. The breasts were well developed and pubic hairs were present, although rather sparse.

Several "café-au-lait" spots were found scattered over the skin. The left eye was exophthalmic and protruded 3 mm more than the right one. The thyroid gland was diffusely enlarged. All epiphyses were enlarged. A moderate genu valgum was noted.

*Laboratory investigations.* Routine blood counts were normal. The urine analysis was likewise normal with sp. gr. 1.028 (urine chromatography was not performed). Serum chemistry: Ca 10.8 mg/100 ml, P 2.3 mg/100 ml, alkaline phosphatase 21.4 Buch units, cholesterol 160 mg/100 ml, urea 30 mg/100 ml, CO<sub>2</sub>-combining power 48 vol%, thymol turbidity 0.05, glycose tolerance test normal. Fat excretion in stools was normal. Urinary excretion of 17-ketosteroids and oestrogens was within normal limits for her age.

*Roentgenological investigations.* All epiphyses appeared widened, cupped and frayed with the typical signs of rickets. The bone age was estimated at 12–13 years. Besides these general signs of rickets she had localized changes widely distributed in the skeleton. The skull was affected on the left side only, with partly sclerotic bone and alternating areas of translucency and increased opacity. Similar changes were found in several bones, mostly on the right side. The bones otherwise appeared normal (Fig. 1). The roentgenological diagnosis was f.d.b. and rickets.

*Treatment and further observations.* She was given vitamin D<sub>2</sub> totalling 1,000,000

units in the course of two days and then sent home. As roentgenograms two months later showed only slight improvement, she was then given five drops daily of AFI-D<sub>2</sub> forte (7000 units per drop) and the dose was later increased to 70,000 units per day. Following this increased dosage her rickets healed satisfactorily. Serum Ca remained normal, but serum P remained low (1.5–3 mg/100 ml), while the alkaline phosphatase activity showed some decrease, although it was elevated most of the time. The Sulko-witch reaction in the urine was + or ++. She has continued to take vitamin D<sub>2</sub> daily since the first hospitalization in dosages between 70,000 and 100,000 units. A supra-condylar osteotomy was performed on both femora at the age of 11, because of increasing deformities of the lower extremities. At the age of 13 she had a grand mal seizure which lasted several minutes. EEG showed an active spike focus in the left temporal region and phenobarbital treatment was started.

The most recent follow-up was when she was 14 years old. She now had a short and broad stature. Height 142 cm (below the 2.5 percentile for age), weight 44 kg. The head appeared enlarged. There was bilateral exophthalmus. The thyroid gland was diffusely enlarged and she had a thyrotoxic appearance with tremor manu and clammy hands. There was slight acne. Pulse rate 120, BP 150/90. The gait was waddling. The lumbar spine was flattened and a thoracic lordosis was prominent. There were flexion contractures of 5° of both knees. Routine blood counts and urine analysis were normal. Serum chemistry: Ca 10.3 mg/100 ml, P 3.1 mg/100 ml, alkaline phosphatase 26.4 Buch units, urea 31 mg/100 ml, PBI 12.6 µg/100 ml, and cholesterol 172 and 162 mg/100 ml. Urea clearance was normal and the creatinine tolerance tests showed 74.2% (normal 55%). Tubular reabsorption of phosphorus (TRP) 54% (normal 85 ± 5%). Urine paper chromatography showed a slight but insignificant generalized increase of amino acids and no definite hyperglycinuria. (Both TRP

and chromatography were performed during treatment.) BMR 140%, 126%, 120%.

The roentgenograms showed no signs of rickets. The epiphyseal lines were closed. The polyostotic lesions had progressed since the first examination. In the skull the lesions were now almost generalized and most marked around the left orbit. New lesions had appeared in the spinal column with compression fractures of T5, L4 and L5, and the ribs now revealed typical dysplastic changes. The lesions had also progressed in the extremities on the right side. Only the metaphyses and diaphyses were affected while the epiphyses were normal. An additional diagnosis of hyperthyroidism was made and treatment with Neomercazol instituted.

CASE 2. N. S., a girl, born 1954. The parents and her two siblings are healthy. An uncle has congenital luxation of the hip. There are no further known cases of congenital anomalies in the family, and especially no other known case of renal glucosuria. Pregnancy, delivery and the neonatal period were normal. From 6 to 12 months age she suffered from eczema and recurrent upper respiratory tract infections, had a poor appetite and was underweight. During the first year the psychomotor development was normal, later she appeared to be slightly retarded. At age 3 years she started to limp, and the right leg was found to be shorter than the left one. Later that year (Dec. 1957) the left tibia was fractured, but healed in good position. In May 1958 the left humerus was fractured and she was treated in a local hospital. At that time it was noted that she had glucosuria with normal fasting blood-sugar levels. Because of a subtrochanteric fracture of the left femur in May 1959, at age 4½ years, she was treated in an orthopedic surgery hospital and was then transferred to the University Pediatric Clinic for investigation of her glucosuria.

*Physical examination.* She was pale, slender and a little irritable. Height 102 cm (10 percentile for age), weight 14.4 kg (2½ percentile for height). She had vitiligo of the





Fig. 1.



Fig. 2.

Fig. 1. G. B. (Case 1). Right radius and ulna. Fibrotic areas predominantly in the right ulna with normal bone structure between the localized lesions.

Fig. 2. N. S. (Case 2). X-rays of both lower legs. The left tibia shows fibrotic areas and scattered patches of irregular rarefaction. The right leg is normal.

skin, but no other discolorations. The left leg was 3 cm longer than the right one and showed a lateral bending.

**Laboratory investigations.** Urine, sp. gr. 1.022, Heller neg. Clinistix +, Benedict reaction +, Sulkowitch +. The positive reactions for reducing substance were found in most specimens, but an occasional specimen taken during fasting was negative. The reducing substance was proved to be glucose by chromatographic studies. Routine blood counts were normal. Fasting blood-sugar level was 85 mg/100 ml, glucose tolerance test began at 95 mg/100 ml, rose to 193 mg/100 ml at  $\frac{1}{2}$  hour and was 70 mg/100 ml after 1½ hours. Serum chemistry: Ca was 9.9–10.1

mg/100 ml, P 2.3–2.7 mg/100 ml, alkaline phosphatase 10.5 Buch units, protein 6.7 g/100 ml with normal electrophoresis curve and thymol turbidity 0.06. Roentgenological examination of the skeleton showed changes characteristic of fibrous dysplasia in the extremities on the left side (Fig. 2), while the skull, ribs, spinal column and the right extremities were normal. The left leg was at least 4 cm longer than the right one. In the metaphyses and diaphyses of the affected bones there were areas of translucency and increased opacity alternating with bone of normal appearance. In the left femur a fracture line was noted. The affected bones were broader than normal. Intravenous

pyelography showed enlargement of the left renal pelvis without signs of stenosis. The calyces were broad and short.

A diagnosis of f.d.b. and renal glucosuria was made and no treatment was begun. Because of increasing bending of the left femur, a corrective osteotomy was performed in Oct. 1959 with a satisfactory result. From July 1959 to Feb. 1960 she was readmitted 3 times to the Pediatric Clinic for further studies. At the physical examinations she was unchanged except for the operative changes. The laboratory investigations showed glucosuria as before, while the fasting blood sugar and glucose tolerance tests were normal on repeated examinations. Serum-chemistry: Ca remained normal, units P 2.6–4 mg/100 ml, alkaline phosphatase 11.6–13.1 Buch units, urea 24–26 mg/100 ml, creatinine 0.5, 1.0–1.3 mg/100 ml, CO<sub>2</sub>-combining power 48.8 vol %, chlorides 105 mEq/L, potassium 4.0 mEq/L. Creatinine tolerance test 66.6 % and TRP 76 %.

Two-dimensional paper chromatography showed a generalized pathological amino-aciduria in four of the five tested specimens. Most increased were leucine, valine, proline and phenylalanine, but cystine, serine, glycine, alanine, threonine and glutamine were also present in pathological amounts. Chromatography of plasma showed no abnormal amino-acid pattern. X-rays did not show further changes.

### Discussion

The diagnosis of f.d.b. is well established in both cases. The first case (G. B.) exhibited the classical triad of McCune-Albright's syndrome. In addition she had vitamin D resistant rickets and hyperthyroidism. N. S., Case No. 2, has polyostotic fibrous dysplasia of the bones; aminoaciduria and renal glucosuria have also been found.

The symptomatology of f.d.b. is well known and will not be reviewed here. The

three chief features of the disease are (1) skeletal lesions affecting only one, a few, many or almost every bone in the body, (2) pigmented patches in the skin, (3) endocrine disturbances manifested by sexual and somatic precocity, especially in the female, and other endocrine disturbances. The skeletal lesions are an essential feature and may or may not be accompanied by either or both of the other two (14). Hyperthyroidism has been reported in several cases (14), cardiovascular anomalies in two cases (14) and diabetes mellitus in one case (15). Symptoms and signs due to mechanical pressure on the eyes and spinal cord, as well as spinal and cranial nerves, are fairly common. To the authors' knowledge, the combination of f.d.b. and renal tubular defects has not been reported previously.

On her first admission to the hospital G. B. had full-blown rickets. According to the history, she had received an adequate vitamin D intake, and she did not respond to ordinary doses of the vitamin. Her serum phosphorus remained low during treatment and the alkaline phosphatase was elevated, although it fell somewhat with treatment. Further studies revealed a reduced TRP, a finding which is in accordance with previous reports of vitamin D resistant rickets. Hyperglycinuria, an inconstant finding in vitamin D resistant rickets (2, 4), was not present in our patient, although it must be admitted that examination for aminoaciduria was not performed until after several years of treatment.

The ultimate cause of vitamin D resistant rickets is unknown. The low serum values and reduced tubular reabsorption

of phosphorus are constant findings. Several authors (2, 4, 17) have stated that this renal tubular dysfunction is the probable cause of the disease. However, Albright (1) has suggested that the reduced reabsorption of phosphorus may be due to hyperparathyroidism (primary or secondary). The latter view was recently supported by Fraser *et al.* (7) who studied the reabsorption of phosphorus during intravenous calcium loading, and found increased reabsorption of phosphorus in the test period in the patients with vitamin D resistant rickets. As pointed out by Harrison in the discussion of Fraser's paper, this does not necessarily exclude the kidneys as the site of dysfunction. Harrison suggests the possibility of the renal tubules possessing increased sensitivity to the parathormone in this disease (7).

The pathogenesis of vitamin D resistant rickets needs further clarification. We find it reasonable to believe that tubular dysfunction plays a significant role either alone or in combination with other metabolic defects. The finding of hyperglycinuria in some of these patients may favor this theory. Inorganic phosphorus is reabsorbed in the proximal tubules. Renal glucosuria and aminoaciduria are also believed to be results of reabsorption defects in the proximal part of the nephron (6). Accordingly the site of the renal defect in both of our cases of f.d.b. can be located to the proximal tubules.

The fundamental biochemical mechanism in these tubular defects is not known, but the finding of Harrison & Harrison (9) that maleic acid inhibits the reabsorption of phosphate, glucose and aminoacids, suggests that a common or closely related

tubular transport system may exist for these substances. This is further supported by the occurrence of renal glucosuria, aminoaciduria and phosphaturia in syndromes of apparently diverse origin such as cystinosis, de Toni-Fanconi syndrome, lead (3) and lysol poisoning (18).

The diagnosis of renal glucosuria in our patient N. S. is based on the following findings. (1) Glucosuria which has been observed on different hospital admissions during 2 years. Glucose has been present in most specimens, but an occasional specimen taken during fasting has been normal. (2) Normal fasting blood sugars and normal glucose tolerance tests on repeated examinations. Simultaneously obtained urine specimens showed glucose in all but one. Hyperglycemia has not been observed. (3) The reducing substance in the urine has been proved to be glucose by chromatographic studies. (4) During the observation period of 2 years there has been no development towards a diabetic state.

The present case does not fulfill all the criteria for the diagnosis of renal glucosuria stated by Marble (13), as some specimens have been normal. Others (10, 11) do not believe that invariable glucosuria is a *sine qua non* for the diagnosis. Reubi (16) has forwarded the hypothesis that there are two distinct groups of renal glucosuria. In both groups there is a defect in the tubules, either separate or as a glomerular-tubular imbalance. The finding of hyperaminoaciduria in our patient also makes it likely that the glucosuria in this patient is due to a tubular defect.

Luder & Sheldon (12) have described a family with four cases of renal glucosuria

and aminoaciduria in three generations. The aminoacids excreted in excess were mainly proline, leucine, and valine, but cystine, lysine, tyrosine, serine, threonine, alanine, glutamine, beta-alanine, alpha amino butyric acid and methionine were also found. The urinary amino acid pattern in Case 2 resembles that described by Luder & Sheldon. It is our opinion that she belongs to this group of patients even though there is apparently no family history.

We do not believe that the finding of renal tubular defects in two cases of f.d.b. give a clue to the etiology of the skeletal lesions. Since these are localized, it is unlikely that they are caused by metabolic disturbances secondary to a renal defect. However, the possibility that the combination of f.d.b. and renal tubular defects might occur by chance is extremely small, considering that both diseases are very

rare ones. We postulate that the tubular defects in our two cases of polyostotic fibrous dysplasia are associated congenital defects, and believe that they may be found more often if they are looked for.

### Summary

The occurrence of renal tubular defects in two cases of fibrous dysplasia of the bones is reported. Case 1 has vitamin D resistant rickets and Case 2 has renal glucosuria and aminoaciduria. The renal defect in Case 2 resembles the syndrome of Luder & Sheldon. According to the present theories on renal function, the site of the renal defects can be located to the proximal tubules in both cases. It is postulated that the renal defects in fibrous dysplasia of the bones are associated congenital lesions.

### References

1. ALBRIGHT, F., BUTLER, A. M. and BLOOMBERG, E.: Rickets resistant to vitamin D therapy. *Amer J Dis Child*, 54: 529, 1937.
2. CHISHOLM, J. J. JR.: Clinical significance of aminoaciduria. *J Pediat*, 55: 303, 1959.
3. CHISHOLM, J. J. JR., HARRISON, H. C., EBERLEIN, W. R. and HARRISON, H. F.: Aminoaciduria, hypophosphataemia and rickets in lead poisoning. *Amer J Dis Child*, 89: 159, 1955.
4. DENT, C. E. and HARRIS, H.: Hereditary form of rickets and osteomalacia. *J Bone Joint Surg*, 38B: 204, 1956.
5. FANCONI, G. and GIRARDET, P.: Familiärer persistierender Phosphatdiabetes mit D-vitamin-resistenter Rachitis. *Helv Paediat Acta*, 7: 14, 1952.
6. FANCONI, G., RÜEGG, J. and DIETERLE, E.: Die "benigne" pyelonephritische und postpyelonephritische Hypertension. *Helv Paediat Acta*, 6: 281, 1951.
7. FRASER, D., LEEMING, J. M. and CERWENKA, E. A.: Über die Handhabung von Phosphat durch die Nieren bei hypophosphataemischen vitamin-D-resistenter Rachitis der einfachen Art und bei Cystinspeicherkrankheit. *Helv Paediat Acta*, 14: 497, 1959.
8. GOLDMAN, L., GORDAN, A. S. and CHAMBERS, E. L.: Changing diagnostic criteria for hyperparathyroidism. *Ann Surg*, 146: 407, 1957.
9. HARRISON, H. E. and HARRISON, H. C.: Experimental production of renal glucosuria, phosphaturia and aminoaciduria by injection of maleic acid. *Science*, 120: 606, 1954.
10. LAMBERT, P. P.: A study of the mechanism by which toxic tubular damage changes the renal threshold for glucose. *Liba Symposium on the Kidney*, p. 79. Boston 1957.
11. LAWRENCE, R. D.: Symptomless glycosuria: differentiation by sugar tolerance tests. *Med Clin N Amer*, 31: 289, 1947.
12. LUDER, J. and SHELDON, W.: A familial tubular absorption defect of glucose and

- amino-acids. *Arch Dis Child*, 30: 160, 1955.
13. MARBLE, A.: Renal glycosuria. *Amer J Med Sc*, 183: 811, 1932.
14. PRITCHARD, J. E.: Fibrous dysplasia of the bones. *Amer J Med Sc*, 222: 313, 1951.
15. PECK, F. and SAGE, C.: Diabetes mellitus associated with Albright's syndrome. *Amer J Med Sc*, 208: 35, 1944.
16. REUBI, F. C.: Glucose titration in renal glycosuria. Ciba Symposium on the Kidney, p. 96. Boston 1954.
17. ROBERTSON, B. R., HARRIS, R. C. and McCUNE, D. J. Refractory rickets: mechanism of therapeutic action of calciferol. *Amer J Dis Child*, 64: 948, 1942.
18. SPENCER, A. G. and FRANGLEN, G. T.: Gross aminoaciduria following a lysol burn. *Lancet*, 1: 190, 1952.
19. WINTERS, R. W., GRAHAM, J. B., WILLIAMS, T. F., MCFALLS, V. W. and BURNETT, C. H.: A genetic study of familial hypophosphatemia and vitamin D resistant rickets. *Medicine (Balt)*, 37: 97, 1958.

Received August 25, 1960

Accepted September 9, 1960

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PROGRESS IN PEDIATRICS

## Toxoplasmosis in Children

### A Study of 83 Swedish Cases

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The interest in toxoplasmosis has been great ever since Wolf, Cowen & Paige (20) in 1939 described the first case in man. Pediatricians have primarily been interested in the congenital form of the disease. The clinical picture of congenital toxoplasmosis is well known. Severe cases show signs of generalized disease: icterus, petechii, enlargement of liver and spleen and involvement of the central nervous system. Most reported cases have not presented symptoms of generalized disease, but instead have had one or several of the symptoms described by Sabin as typical of congenital toxoplasmosis (convulsions, chorioretinitis, hydrocephalus, and intracerebral calcifications). Most cases have had a fatal outcome or a very poor prognosis with invalidizing symptoms and mental retardation. Gard, Magnusson & Hagberg (4) and Hedenström (7) have, however, described cases with benign course and normal mental development.

The acquired type of toxoplasmosis was first described in 1941 (12). The cases which were initially described all had septic fever, and many had a fatal outcome. Later, mild cases were observed and

it seems that most of them ran a slight or a subclinical course. It is probable, however, that the disease always starts as a generalized infection, even though symptoms of this might be missing. In Scandinavia the most common clinical symptoms seems to be lymphadenopathy with or without fever often resembling infectious mononucleosis (1, 3, 5, 17, 18). Few cases of acquired toxoplasmosis have been described in children. Sabin (14) was the first to describe a case in a child (1941), a six-year-old boy who had meningo-encephalitis with a fatal outcome. Siim (18) has reported some cases with febrile lymphadenopathy in boys.

The great variations in the clinical picture of toxoplasmosis have thus been amply demonstrated. Yet the epidemiology is poorly understood. In planning a study in this field, we began with a survey of cases of toxoplasmosis diagnosed in Swedish children during the period 1951 to 1959.

#### Material and Methods

The case material consists of 83 children with serological signs of an actual or recent y

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Fig. 1. A toxoplasma test, X

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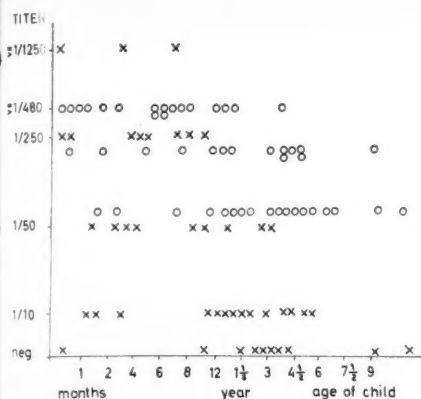


Fig. 1. Antibody titers in children with congenital toxoplasmosis related to age. O = titer in dye test, X = titer in complement fixation test.

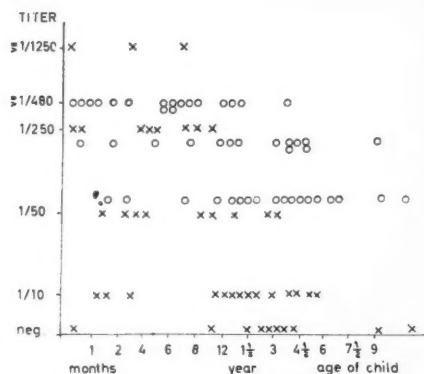


Fig. 2. Antibody titers in mothers of children with congenital toxoplasmosis, related to the child's age. O = titer in dye test, X = titer in complement fixation test.

subsidized toxoplasmosis. There was evidence that in none of the cases were the antibodies demonstrated passively transferred. The cases came from various parts of Sweden, from Kiruna in the north to Lund in the south. Fifty-one children lived in urban and 33 in rural districts.

A summary of the clinical findings was obtained through the courtesy of the heads of various pediatric departments. Serological investigation using the dye test (DT) and the complement fixation test (CFT) were performed at the State Bacteriological Laboratory, Stockholm (8, 15).

In the following presentation the material is divided into three groups: I, congenital toxoplasmosis; II, acquired toxoplasmosis; III, cases uncertain if congenital or acquired.

### I. Congenital Toxoplasmosis

The material comprises 62 cases of congenital toxoplasmosis. The diagnosis in most cases was based on a typical clinical picture and elevated titer values for antibodies against toxoplasma. Fifty-six of these children had high antibody titers

against toxoplasma ( $DT \geq 1/1250$  and/or  $CFT \geq 1/120$ ). The six remaining cases with lower titer values were all below one year of age, which indicated that the infection must have been rather recent and probably congenital. Fig. 1 illustrates all titer values obtained from these congenital cases and includes results of different measurements in the same patient. Blood specimens from the mothers were obtained in 49 cases and they all showed elevated titer values even several years after the birth of the patient (Fig. 2).

Some of the findings in this study are summarized in Table 1 and Fig. 3. Fifty-eight patients showed one or several of the symptoms described as typical of congenital toxoplasmosis. As these symptoms have been repeatedly reported in the literature, we will not go into details here. Table 2 summarizes some findings in twelve patients observed during the neonatal period. These represent very severe cases with generalized disease and often fatal



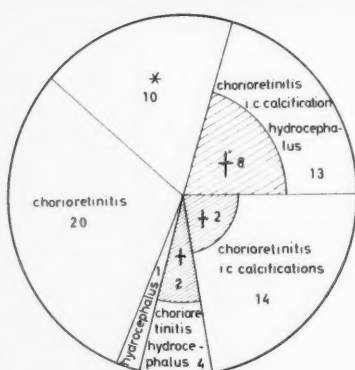


Fig. 3. Symptomatology and lethality in 62 cases of congenital toxoplasmosis. \* without chorioretinitis, hydrocephalus or intracerebral (i.e.) calcifications. Seven with epilepsy, eighth mentally retarded.

outcome. The following case might be mentioned as an example of this fulminating type of congenital toxoplasmosis.

H.G. born Jan. 7, 1959. Third child of healthy parents. Some episodes of bleeding in the seventh month of gestation, born one month before term. Birth weight 2700 g. At birth, the child had petechii and thrombocytopenia (min. value 12,000/mm<sup>3</sup>). After a couple of days, the child's condition

deteriorated, she had a high pitched cry and developed generalized convulsions, a bulging fontanelle and icterus. The child had bilateral uveitis and X-ray of the skull showed calcifications around the ventricles. There was a marked anemia, bilirubinemia and gammaglobulinemia. The cerebrospinal fluid had a very high protein content and increased level of bilirubin and a slight pleocytosis. Serological examination of serum revealed a DT of 1/1250 and CFT 1/120. The titers in the mother were at that time DT 1/1250 and CFT 1/480. Ten months later mother's DT was 1/1250 and CFT 1/120. Toxoplasma was isolated from cerebrospinal fluid collected on the ninth day of life. The urine was positive for protein but the sediment revealed nothing pathological. In smear from the bone marrow, no megacaryocytes were observed, but the morphology was otherwise normal. The child's condition deteriorated in spite of treatment with sulfamerazin and aralen. He succumbed in the sixth week of life. Post-mortem examination showed very extensive encephalitis of the type repeatedly described in congenital toxoplasmosis.

In contrast to this fulminating type of congenital toxoplasmosis, the following case histories of two identical twins might be cited.

TABLE 1. Symptoms and occurrence of prematurity and deaths among 62 patients with congenital toxoplasmosis.

Symptoms	Total number	Prematures	Dead	Epilepsia	Mentally retarded
Chorioretinitis Intracerebral calcifications Hydrocephalus	13	6	8	3	5
Chorioretinitis Intracerebral calcifications	14	2	2	7	5
Chorioretinitis Hydrocephalus	4	—	2	—	1
Chorioretinitis	20	2	—	4	7
Hydrocephalus	1	—	—	—	—
Without the above symptoms	10	1	—	7	8
Total	62	11	12	21	26

TABLE 2. *Clinical picture of congenital toxoplasmosis in the neonatal period.*

	Total number	Chorioretinitis	Intracerebral calcifications	Hydrocephalus	Enlargement of liver and spleen	High content of protein in liq. cerebrospinal	Lung symptoms	Toxoplasma organisms found
Surviving	5	5	5	3	1	4	—	—
Dead	7	7	7	5	6	6	7	7
Total	12	12	12	8	7	10	7	7

TABLE 3. *Condition at follow-up in 50 surviving patients with congenital toxoplasmosis.*

Follow-up period, years	Number of patients with symptoms of							No sequelae
	Chorioretinitis, epilepsy, mental retardation	Chorioretinitis, epilepsy	Chorioretinitis, mental retardation	Epilepsy, mental retardation	Epilepsy	Chorioretinitis	Mental retardation	
<1	—	1	1	—	—	—	1	—
1-2	1	1	—	3	1	4	1	—
3-4	—	1	1	5	2	3	4	1
5-6	1	1	3	—	—	2	3	1
7-8	—	1	1	1	—	—	1	—
9	—	4	1	—	1	—	—	—
Total	2	9	7	9	4	9	10	2

Sachs Children's Hospital, nos. 270/49 and 271/49. Boys, three years of age. Parents and two older siblings healthy. Pregnancy, delivery and neonatal period normal. Normal development. Strabismus observed at the age of six and twelve months respectively. Examinations showed chorioretinitic scars—the mirror-image of each other in the right and left eye respectively. Otherwise the findings were normal. X-ray of the skulls were normal. The IQ was 91 for both boys (not tested under optimal conditions). Serology: one boy DT = 1/100, the other DT = 1/200 and the mother DT = 1/200.

Fifty patients were alive when last examined. Table 3 summarizes some of the findings at that time. It is probable that in most cases the toxoplasmosis was

healed, but the residual symptoms were often severe. The mental retardation observed in 30 patients was of moderate degree corresponding to that of children who can benefit from teaching in special schools (IQ ca. 50-70, sometimes up to 80). Twenty-four children were reported as having convulsions, mostly of the grand mal type. Electroencephalography was done in five of these patients and showed evidence of grand mal epilepsy. The convulsive disorders seemed to respond to treatment in the usual way. At least 27 children had scars after chorioretinitis, but in no case was the vision severely impaired on both sides. Most children had a combination of several symptoms, which

increased their handicap. It is interesting, however, that two children had no residual symptoms and seemed normally developed.

In none of the cases were symptoms of toxoplasmosis reported during the mother's pregnancy. It should also be mentioned that at least seven mothers have subsequently given birth to normal children who have had no signs of congenital toxoplasmosis.

## II. Acquired Toxoplasmosis

In 17 patients 10 boys and 7 girls high antibody titers ( $DT \geq 1/1250$ ,  $CFT \geq 1/120$ ) were considered to be due to acquired toxoplasmosis. Five of these children showed a clinical picture typical of toxoplasmosis, and in 12 the actual symptoms were probably not caused by toxoplasmosis which seems to have run a subclinical course in these cases.

Five children were below five years of age. Their mothers had negative serological tests. The rest of the children were over six years of age and their high antibody titers made a diagnosis of congenital toxoplasmosis less probable (see below for discussion). A significant rise (more than four times) in the titers were observed in five cases. *Toxoplasma* was isolated from a lymph node in one case.

Three girls and two boys, ages 6 to 13, had lymphadenopathy as the main symptom. The lymph nodes of the neck as well as in the axillae and/or groins were enlarged. The disease had a benign course. Three children had moderate fever for a couple of days and two were afebrile. A blood differential count showed relative lymphocytosis in four cases and eosinophilia of 5% or more in three cases. The sedi-

mentation rate was 30 mm/l hour in one case, but below 15 mm/l hour in the rest. The bone marrow was examined in two cases and showed hyperplasia of the reticulum cells and increased number of plasma cells and lymphoid cells. In two cases biopsy from an enlarged lymph node revealed a histopathological pattern that is often seen in toxoplasmosis, i.e. enlarged reticulum cells and increased number of eosinophile cells and plasma cells.

K.B., girl born on Sept. 24, 1947, was well until August, 1957, when she became acutely ill with fever, cough and rhinitis (Fig. 4). Afebrile after four days. Ten days later she saw a yellow spot before her left eye and the vision was impaired. An ophthalmologist (Huggert) found an acute chorioretinitis in the left eye. The patient was admitted to a pediatric clinic, where she presented enlarged lymph nodes in the neck and the axillae. Histopathological examination of an excised lymph node showed evidence of a slight chronic infection and toxoplasma were isolated from the lymph node. She had no anemia but a differential count showed a slight eosinophilia (8%). X-ray of the lungs and skull revealed normal findings. ECG and EEG were normal. Smears from the bone marrow revealed a hyperplastic reticulum and plasma cells were numerous. Serology: one month after onset of symptoms DT was 1/250, CFT 1/60 and one month later DT was 1/1250 and CFT 1/120. The mother had negative serological tests.

This case is of special interest because she developed chorioretinitis. Despite the great interest in the investigation of toxoplasmic eye disease that has been shown in different parts of the world for years, only a few cases are known where uveitis was a symptom of clinically recognized acute acquired toxoplasmosis (6, 9, 10, 11, 19). In this case, as in one published by Kayhoe and coworkers (10),

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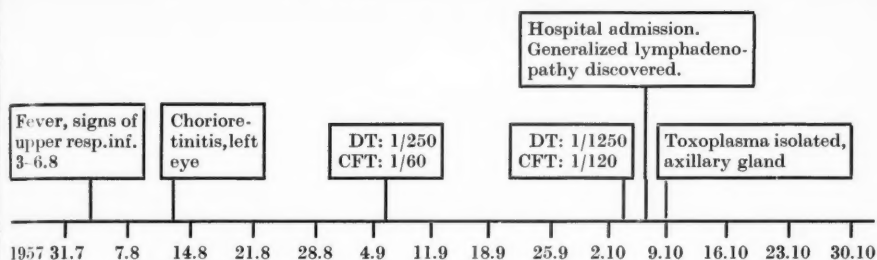


Fig. 4. Acquired toxoplasmosis in 10-year-old girl with chorioretinitis and lymphadenopathy. Maternal DT and CFT negative.

parasites were isolated from other areas of the patient. It therefore appears likely that the eye lesion was the result of ocular infection with toxoplasma.

In an eight-year-old boy admitted to a department of child psychiatry, a routine serological investigation revealed rising titers against toxoplasma (DT rose from 1/250 to 1/6250, CFT from 1/60 to 1/480). The boy was afebrile and had rectal bleeding. Blood examination showed anemia, elevated gamma-globulin and slight eosinophilia (9%) and smears from the bone marrow reticulohyperplasia and increased number of eosinophile cells and plasma cells. Lumbar puncture and eye examinations revealed normal findings. Obviously this was a case of acute subclinical toxoplasmosis that was diagnosed occasionally.

In eleven other children between 3 and 14 years of age, high antibody titers were found. The children had symptoms which seem not to have been caused by toxoplasmosis. Two cases are of special interest because the symptomatology was in some respects like that in toxoplasmosis.

Two seven-year-old boys had lymphadenopathy and both had DT 1/1250 and CFT 1/120. They were first classified as cases of acquired toxoplasmosis. In one of the boys a lymph node was removed which revealed evidence of tuberculosis. This diagnosis was

verified by a guinea pig inoculation. In the other boy, who, in addition to the lymphadenopathy, had fever, leucocytosis and enlarged spleen, the Paul-Bunnell test was positive, indicating an infectious mononucleosis.

In the last 12 cases the high antibody titers against toxoplasmosis indicate an acute or recently subsided toxoplasmotic infection, obviously of subclinical nature. The cases demonstrate that care must be taken when correlating high titers, to any clinical symptoms that may be present

### III. Cases Uncertain if Congenital or Acquired

High antibody levels were demonstrated in four patients in whom it was impossible to decide if the infection was congenital or acquired. Two children were four years old, the others six and eight respectively. The mothers had slightly elevated antibody titers. Two of these children had convulsive disorders, one showed a slow speech development. The last had strabismus and a behavior disorder, probably of psychogenic origin. None of these patients had hydrocephalus, intracerebral calcifications or signs of chorioretinitis.

### Discussion

The diagnostic criteria in toxoplasmosis have been widely discussed (8, 9, 13, 16). As in all infectious diseases, the laboratory diagnosis should be based on the demonstration of the organism *and* a specific antibody response. The isolation of toxoplasma is, however, difficult. Material has to be obtained through biopsy (preferably from spinal fluid or involved lymph nodes). In direct smear or histopathological preparations it might be impossible to differentiate toxoplasma from other parasites. To isolate toxoplasma, inoculation of mice is primarily used. It should be kept in mind that human strains are not always pathogenic to mice. However, specific antibodies always develop in the mice.

It is thus often necessary to base a diagnosis on the results of clinical and serological investigations alone. The clinical picture is not pathognomonic, as, e.g. "inclusion body disease", may present the same clinical pattern as congenital toxoplasmosis (13) and mononucleosis sometimes cannot clinically be differentiated from toxoplasmic lymphadenopathy (1, 8, 18).

The specificity of the serological reactions seems beyond doubt after the vivid discussion of recent years (2, 9). In newborns, elevated antibody titers which are not passively transferred suggest a congenital infection. If the children are not examined until later in life, it is often difficult to know whether the disease was pre- or postnatal. In congenital toxoplasmosis, the mothers show elevated antibody levels for many years or even decades. If the mother is negative, the in-

fection in the child is probably acquired. If she is positive the child can have either a congenital or an acquired disease. In differentiating between these two conditions attention should be given to the time of onset of symptoms, as the same symptom can be present in both types (chorioretinitis and meningoencephalitis).

Significantly increasing antibody titers suggest actual infection. It is more difficult to evaluate unchanged high titers. The titers in the DT usually decrease within 6-7 months after an acquired infection, but cases with titers remaining high and unchanged for years have been observed (8). In cases of unchanged high titers the diagnosis of an actual infection can be established only when there is a typical clinical picture with, e.g. lymphadenopathy and typical changes in the blood and/or bone marrow and lymph nodes or when the parasite can be isolated from infected material.

Unfortunately there is no standardized procedure for serological investigations in toxoplasmosis and the antigens used seem to vary considerably (6). The titer values in this series are rather high compared with those of most other investigators. This is especially true of the titers in the CFT, as earlier discussed by one of us (6). In individual cases, the titers in DT often are more constantly elevated and higher than those in CFT, which corresponds to earlier findings. This is, however, not true in some cases where the titers in CFT are higher than those in DT.

Our findings in congenital toxoplasmosis (Tables 1 and 2) resemble those in earlier series (4, 8, 9, 20). Cases with neonatal disease usually run a severe and often fatal course (Table 2). In children who

developed symptoms later, the most common was chorioretinitis, but this was not present in all cases. The frequency of various symptoms is somewhat lower in this than in earlier series. We have in this study some mild cases also and two without residual symptoms. Such mild cases have earlier been reported (7) and might be more common than hitherto believed. We do not know why the clinical course was benign in these cases and can only speculate on the role of the infectious dose, the virulence of the infecting organism and the protecting role of the transferred antibodies from mother to fetus.

In our patients with acquired toxoplasmosis two facts were of interest. Thus in an eight-year-old girl with obviously acquired toxoplasmosis uveitis was the dominating symptom. Further, 11 of 17 cases of acquired toxoplasmosis had a subclinical course which indicates that subclinical acquired toxoplasmosis is common in children as well as in adults.

As yet, no mother had two successive children with congenital toxoplasmosis. Thus there is no need to advise against further pregnancies as soon as there are

no clinical signs of toxoplasmosis in the mothers and when the titers have begun to decrease.

### Summary

A report is given of 83 cases of toxoplasmosis in childhood. The series represents most cases diagnosed in Sweden during the period 1951 to 1959. The diagnostic criteria and the differential diagnosis between congenital and acquired forms of the disease are discussed. Sixty-two patients were considered to have the congenital disease and 17 the acquired. In four patients it was impossible to determine if the disease was congenital or acquired. The series of congenital cases comprises some with none or very few symptoms, and normal development. Acquired toxoplasmosis in childhood seems to give the same clinical symptoms as in adults. The most common symptom is lymphadenopathy, with or without fever. One patient had acute chorioretinitis. The disease often runs a mild course and subclinical infections are probably not uncommon in childhood.

### References

1. ADAMSON, C. A., HEDENSTRÖM, G., HULDT, G. and LINDAHL, J.: Synpunkter på förvärvad toxoplasmos i anslutning till 13 fall. *Svensk Läkartidn.*, 54: 2717, 1957.
2. BEKKAL, MAS P.: Het Problem van de Specificitet van de Reactie van Sabin en Feldman bij de Diagnostiek van Toxoplasmos. Groen, J. J. & Zoon, N.V. Leiden 1959.
3. GARD, S. and MAGNUSSON, J. H.: A glandular form of toxoplasmosis in connection with pregnancy. *Acta Med Scand.*, 141: 59, 1951.
4. GARD, S., MAGNUSSON, J. H. and HAGBERG, E.: Congenital toxoplasmosis. *Acta Paediatr.*, 41: 15, 1952.
5. GRÖNROOS, P.: Studies on toxoplasma and the serology of toxoplasmosis. *Ann Med Exp Fenn.*, 33: 310, 1955.
6. HABEGGER, H.: Toxoplasmos humaine, mise en évidence des parasites dans les milieux intra-oculaires (humeur aqueuse, exsudat rétro-rétinien). *Arch Ophthal (Par)* 14: 470, 1954.
7. HEDENSTRÖM, G.: On the variability of the clinical course of congenital toxoplas-

- mosis with special reference to the occurrence of mild cases. *Acta Paediatr* 46: 579, 1957.
8. HULDT, G.: The dye test and complement fixation test in toxoplasmosis. A comparative investigation. *Acta Pathol Microbiol Scand*, 43: 141, 1958.
  9. — Acquired Toxoplasmosis. Human Toxoplasmosis. Munksgaard, Copenhagen 1960.
  10. JACOBS, L., FAIR, J. R. and BICKERTON, J. H.: Adult ocular toxoplasmosis. *Arch Ophthalm*, 52: 63, 1954.
  11. KAYHOE, D. E., JACOBS, L., BEYE, H. K. and McCULLOUGH, N. S.: Acquired toxoplasmosis; observations on two parasitologically proved cases treated with pyrimethamine and triple sulfonamides. *New Engl J Med*, 257: 1247, 1957.
  12. MAGNUSSON, J. H.: Toxoplasmosens klinik. *Nord Med*, 45: 344, 1951.
  13. PINKERTON, H. and HENDERSON, R. G.: Adult toxoplasmosis. *JAMA*, 116: 807, 1941.
  14. Recent Advances in Paed. Ed. D. GAIRDNER. J. & A. Churchill Ltd, London 1958.
  15. SABIN, A. B.: Toxoplasmic encephalitis in children. *JAMA*, 116: 801, 1941.
  16. SABIN, A. B. and FELDMAN, H. A.: Chorioretinopathy associated with other evidence of cerebral damage in childhood. *J Pediatr*, 35: 296, 1949.
  17. SABIN, A. B., EICHENWALD, H., FELDMAN, H. A. and JACOBS, L.: Present status of clinical manifestations of toxoplasmosis in man. *JAMA*, 150: 1063, 1952.
  18. SAXÉN, E. and SAXÉN, L.: The histological diagnosis of glandular toxoplasmosis. *Lab Invest*, 8: 386, 1959.
  19. SHIM, J. CHR.: Toxoplasmosis acquisite lymphonodosa. Clinical and pathological aspects. *Ann NY Acad Sci*, 64: 154, 1956.
  20. WISING, P. J.: Akut adult toxoplasmos med lymphadenopathi och chorioretinit. *Nord Med*, 47: 563, 1952.
  21. WOLF, A., COWEN, D. and PAIGE, B. H.: Toxoplasmic encephalo-myelitis new case of granulomatous encephalomyelitis due to a protozoan. *Amer J Path*, 15: 657, 1939.

Received Sept. 1, 1960

Accepted Oct. 1, 1960

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SUMMARY OF SUPPLEMENT

## A Longitudinal Study of Electrocardiographic Intervals in Healthy Children

by ROBERT W. McCAMMON

(Supplement 126)

Electrocardiographic interval measurements in the three standard limb leads were made on 4993 tracings taken serially from 214 healthy children from one month of age to their present ages as part of the study of human growth and development of the Child Research Council. Group standards were computed for P-R, Q-S, and Q-T interval duration in the three standard limb leads and for pulse rate and Q-Tc in lead II. Individual subjects were presented against the group background to demonstrate developmental changes in healthy subjects from infancy to maturity. Characteristic patterns of stability and instability were discussed together with their clinical applications. The following conclusions were drawn:

1. Pulse rate is a very unstable measurement during at least the first two years of life, becoming more stable after that but still showing both short-term and long-term fluctuations without obvious cause.

2. The P-R interval is a very stable measurement within the group range for any individual in health.

3. Changes in the P-R duration of more than 0.02 second on records from the same individual within any one year were not seen in the absence of illness or obvious change in pacemaker.

4. Changes in the duration of the P-R interval which occur in the absence of disease are usually confined to one lead, while those associated with cardiac abnormality occur simultaneously in all three leads to approximately the same degree.

5. No linear relationship could be demonstrated between P-R duration and pulse rate either for the individual or in the group data. A highly significant U-shaped dependence was found between P-R duration and pulse rate for the group data. It was speculated that this dependency resulted from some factor or factors not measured.

6. No relationship could be found between the duration of the P-R interval and heart size at a single age or between P-R duration and changes in heart size with body growth.

7. Group data for the Q-S interval duration strongly suggest that the duration of this interval is related to physical size and therefore to heart size, though conformity to a pattern corresponding to established anatomic growth curves is not universal.

8. The frequent occurrence of Q-S durations in excess of 0.10 second after 12 years of age makes this an unacceptable upper limit of normal duration.

9. The Q-T interval shows partial dependence on the pulse rate both for the group data and within the individual, but this is not uniform or predictable.

10. The corrected Q-T interval, using the square root ratio of Taran, shows as wide a range for the group data as is found for the uncorrected Q-T.

11. Individuals in health characteristically fluctuate widely through the group range for both the Q-T interval and for the corrected Q-T interval.

12. The range for the corrected Q-T interval duration extends upward far enough so that a minimum of 25 % of the data falls above the previously published upper limits of normal.

13. The equation used for correcting the Q-T duration for pulse is inadequate for very fast or very slow pulses.

14. There is a temporary rise in Q-T duration between the ages of 18 and 22 years which occurs in most if not all subjects independent of rate changes.

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PROCEEDINGS OF PEDIATRIC SOCIETIES

Swedish Pediatric Society

Meeting Sept. 29, 1960

**Trevor P. Mann: Neonatal cold injury due to accidental exposure to cold**

In Great Britain neonatal cold injury is a not uncommon disorder with a high mortality. In Brighton in the last six years or so 16 examples have been admitted to the Children's Hospital and no less than eight have died. Cases generally arise after home confinements in winter when outside air temperatures fall below freezing point. An unknown but appreciable number of newly-born babies die in this way each year. This waste of life, which is almost entirely preventable, is now receiving considerable attention. The nature of the disorder frequently goes unrecognised, the predominant aetiological role of exposure to cold being overlooked. The condition may be incorrectly labelled sclerema neonatorum or haemorrhagic pneumonia, to mention two recurring misdiagnoses. Increasing apathy and food refusal are the most constant presenting features, but the infant does not look ill. In a number of cases coldness to touch has been noted for several days by the mother, but she may have difficulty in arousing the interest of her medical attendants in this important physical sign. In none of the series has the severity of the hypothermia been realised before admission. Oliguria is a frequent accompaniment. Constant clinical findings are severe hypothermia (all cases below 32°C on admission; three less than 27°C), oedema of the extremities and striking skin erythema, the latter largely contributing to the misleading appearance of well-being. A purulent nasal discharge occurs commonly but sometimes not until rewarming is under way. The predominance of male infants in this

series is striking (12 of 16 cases, males). Prematurity is not important, there being only three small babies with birth weights of 5½ pounds or less amongst the 16 cold babies. Twelve of the 16 cases were admitted in January, February and March, generally the coldest months of the year. A noticeable feature is that the occurrence of cases generally coincides with spells of severe weather when minimum air temperatures fall below freezing point. Social conditions are not always bad; a comfortable and well-provided home may still be cold in winter, especially at night-time. Predisposing factors include asphyxia at birth, birth injury and infection. Food refusal and bathing in a cold environment play a contributing role in a frigid home. A prognosis is usually possible within a few hours of commencing rewarming and these are all considered ominous signs: apnoeic attacks, persisting oliguria, hardening of oedema and bleeding from the mouth and nose, the latter usually being accounted for by pulmonary haemorrhage.

A post-mortem examination was carried out on the fatal cases. There were no specific features to relate an infant's death to cold injury. If at necropsy massive pulmonary haemorrhage, mild ascites and oedema of the extremities are found, then a careful scrutiny of clinical data is desirable for features suggestive of cold injury, especially during the winter months.

The best method of treatment is still undecided. Therapy may be considered under three headings.

*Re-warming.* The majority of cases fall into the chronic or prolonged group (hypo-

thermia for over six hours), for which slow or passive rewarming is favoured. This is best carried out by obviating heat loss and allowing the infant to warm up through the processes of metabolism.

*Feeding.* One of the first effects of rewarming is to accelerate metabolism and to increase demands for glucose. Profound hypoglycaemia, if not present initially, may be precipitated if nourishment is withheld. Where the hypothermia is prolonged, as

just defined, 15% glucose by the intragastric is being tried.

*Drugs.* As an infective process may be difficult to recognize initially and as septic manifestations often appear during resuscitation, antibiotic therapy should be given early. Cortico-steroid treatment is advocated by some workers, but the rationale is not understood. Antibiotic cover is imperative if this hormone is used.

#### Meeting Dec. 2, 1960

##### *Karl-Magnus Herrlin: Frequency and prognosis in flexor spasms of infancy*

At Kronprinsessan Lovisas Barnsjukhus during the ten year period, 1950-1959, 693 children (390 boys, 303 girls) under the age of four years were examined because of epilepsy. Of these, 48 cases (26 boys, 22 girls) had flexor spasms ("Nick- und Salaamkrämpfe", "spasmes en flexion"). In this series of 693 children, 290 had the onset of seizures before one year of age, 45 of whom (15.5%) had flexor spasms. Of all the 48 infants with flexor spasms, 19 (40%) have died, 21 (44%) are severely mentally retarded and 8 (16%) are moderately retarded. The case material will be published.

##### *Å. Gyllenswärd and S. Malmström: Cerebrospinal fluid in premature infants*

Studies of the cerebrospinal fluid with regard to cells, protein and bilirubin were performed in thirty-six prematures. The babies' general condition, their hemoglobin, red and white blood cells, serum protein and serum bilirubin were also followed. Lumbar punctures were generally made on the third, eleventh and thirty-ninth days of life (I, II and III respectively in the table). The mean birth weight was 2012 g. On the average,

two of the tests from each child could be used for evaluation (in ten cases all three). The results regarding cells and protein are shown in the following table. Cell content was not correlated to the birth weight, while protein was negatively correlated. No correlation was shown between the bilirubin in the serum and that in the spinal fluid. Nor between the general condition and the level of serum bilirubin, content of cells or protein. The highest value noted for the bilirubin was 19 mg %.

##### *E. Rabo: Triple vaccination, methods and secondary effects*

The most suitable area for a subcutaneous injection has been investigated in several groups of 200 children who received their first and second injections in two different sites. The mother's opinion as to which injection caused the child and the mother the most trouble, and anxiety has been evaluated. The majority of mothers regard the second injection as causing more trouble than the first one. This source of error was avoided by alternating the site of the first injection in every other child. The best site of injection was the pectoral region. If the incidence of complaints is put at 1.0 for injections over the pectoralis muscle, it is 4.2 in the sup a-

Puncture	I no. = 21		II no. = 28		III no. = 23	
	Cells/3 mm <sup>3</sup>	Protein/mg %	Cells/3 mm <sup>3</sup>	Protein/mg %	Cells/3 mm <sup>3</sup>	Protein/mg %
Mean	27	150	20	110	17	86
Median	22	149	15	99	12	76
Spread	4-112	57-292	3-56	74-189	2-70	55-166

spinatus fossa, 2.6 in the deltoid region and 1.7 in the gluteal region, which thus is the second best spot of injection. The subcutaneous and intramuscular injections in the gluteal area were compared in one experiment. Intramuscular injections caused considerably more trouble than subcutaneous ones. Thus, 49 of 200 children had no complaints after the subcutaneous injection, while only eight had no trouble following the intramuscular injection. In a smaller series, shallow and deep subcutaneous injections were compared. After 4-6 weeks, a palpable infiltrate was noted in 40% of the shallow injections but only 3% of the deep ones. Abscess formation was 10 times more frequent after the shallow injection, which therefore ought to be avoided. Finally subcutaneous injections of Cutter vaccine and Swedish triple vaccine were compared. The incidence of complaints was equal.

**DISCUSSION:** *H. O. Mossberg:* The mothers in Västervik were somewhat hesitant about triple vaccination in 1956 when the well-baby clinic was started in the hospital there. Some unusually strong local and general reactions to the ordinary dose of 1 ml of vaccine were observed. Since 1958, a first injection of 0.7 ml has been given (deep subcutaneously supra spinam), the second and the third injections have been with 1 ml, or somewhat less, if the reaction to the previous injection had been too strong. The case material comprises slightly more than 300 children. Two injections have been given to 97.8% of the children, and three to 93.5%. Moderate reactions (i.e. fever = 38°C for 2-3 days) possibly some sluggishness and local tenderness were observed after the first injection in 4%, after the second in 17.7% (78% injected with 1 ml) and after the third injection in only 5% (73% given 1 ml). Total frequency of moderate reactions was 8.9%, which is higher than earlier reported in any Swedish series. A dose of 0.5-0.7 ml has given local abscess formation in 0.8% compared with a frequency of 2.4% following the dose 0.8-1 ml. Of the individuals with slight general reactions 1.3%

developed abscesses, but 7.1% of those with moderate reactions later had abscess formations. It is probable that these doses convey somewhat lower immunity than full doses but the lower frequency of side effects might favour my approach.

#### *B. Eckerberg:* A case of prenatal mycosis

The patient, an infant girl, six weeks premature, weighing 2800 g, was born after a normal delivery. On the second day of life she developed convulsions and on the fourth day diarrhea. On admission to the pediatric ward the sixth day, she was sluggish, microcephalic, and had a fluctuating tumor in the neck, the size of a walnut. Puncture of the tumor revealed pus from which *E. coli* were cultured. The patient later developed profuse diarrhea, vomiting and convulsions and died on the 25th day of life. On post-mortem examination a cystic abscess was found connected to the esophagus and containing fungus and bacteria. Intracerebral calcifications were seen in the atrophic brain parenchyma, especially around the ventricles. Fungus colonies with appearance of *Candida albicans* were also demonstrated in the periphery of these calcifications. No fungi were found in other organs. This is probably a case of early prenatal infection with *Candida albicans*. It probably started as an esophagitis with abscess formation and later hematogenous spread to the brain. This case is analogous to one described by Burry in 1957 (*Arch Dis Child* 32: 161).

#### *C. Lingén, B. Wengle and H. Boström:* Metabolic screening of urine for inborn errors of metabolism in a pediatric material

During the last year, a series of simple chemical reactions and paper chromatographic methods have been applied to urine specimens from all patients (1500) of the pediatric department of Karolinska sjukhuset, Stockholm. Only two cases of "inborn errors of metabolism" were found; one case of porphyria and one of cystinuria. This agrees well with the expected low incidence of these disorders. Some interesting facts about the excretion of hydroxyproline and of ethanol-

amine together with beta-aminoisobutyric acid combined with lactosuria were observed. Thus an increased excretion of the aminoacid hydroxyproline was found in 50% of cases with hyperbilirubinemia not associated with immunological factors. Patients with hyperbilirubinemia caused by blood-group incompatibility (Rh or ABO) did not seem to excrete pathological amounts of hydroxyproline.

### *Symposium on poisoning*

#### **Introduction.—Bengt Karlsson: Poisoning cases of current interest**

During 1960 a Poison Control Center was established at the pediatric clinic, Karolinska sjukhuset, Stockholm. Although the center has functioned primarily as an information center it is also concerned with education, research and treatment. The cases presented here have been selected to illustrate poisoning problems which have been referred to the center.

#### **Bengt Karlsson: Saffron poisoning**

An 18 year old woman was admitted to the Visby hospital 15 hours after she had started to complain of abdominal pain with diarrhea and vomiting. She was in poor condition with icteric skin and sclerae and with cyanotic lips. Blood pressure was 160/110; pulse 120. The abdomen was distended and there was a marked tenderness under the right costal margin. Laboratory findings included Hbg 40%, Rbc 1.5 million, Wbc 20,800, hematuria. The patient became anuric and later she became unconscious but before that confessed that the evening before admission she had eaten saffron because of a suspected pregnancy. She died a few hours after admission. Post mortem examination revealed generalised icterus, pronounced pulmonary edema and swelling of the parenchymatous organs. The histopathology showed pronounced renal tubular damage. Saffron has been used for many years as an abortion provoking agent. The lethal dosage is said to vary between 3 and 12 g.

#### **C. Thorén: Poisoning by furniture polish**

A 1½ year old boy drank an unknown quantity of furniture polish. Half an hour later, the boy vomited and the mother provoked further vomiting. He later became sluggish, hypotonic, and tachypneic. When he arrived at Kronprinsessan Lovisas Children's Hospital 1¼ hours after the ingestion, he was unconscious, pale, cyanotic and pulseless. He was immediately put in the respirator, but the cyanosis persisted in spite of adequate ventilation. ECG showed a shifting pace-maker, ventricular extra systoles, and progressive increasing lowering of ST-T segments as in severe myocardial ischemia. Later bradycardia developed which changed into ventricular fibrillation following which the patient died. Post-mortem examination revealed signs of moderate amount of aspiration of small hemorrhages in the bronchi. Routine chemical analysis for poison in the stomach contents, the blood and various organs was negative.

Two-thirds of all the many different furniture polishes contain 25–75% vanolen, a petroleum derivative resembling kerosene. In this case, it contained 17% vanolen, 3% xylol, 30% spider oil, 1% risinic oil, 2–3% aromatic oils and water.

The polish was tested for toxicity at the State Pharmacological Laboratory. It was atoxic to mice when given orally in dosages up to 18 ml/kg body weight. Inhalation of pure vanolen and the actual polish by mice and guinea-pigs in "mist chambers" did not cause any lung lesions.

Injection of kerosene in the trachea has, however, been shown to cause death in a couple of minutes through hemorrhagic edema and membrane formation in the lung alveoli. Pneumonia after kerosene intoxication has been known for a long time and has been demonstrated by X-ray examination after one hour. Aspiration of kerosene in animals and men has been shown to cause lung lesions. In text-books the provoking of vomiting and the use of emetics after kerosene ingestion is emphatically warned against. Stomach lavage should be done



only after intubation. Olive oil can be given orally to decrease the resorption.

Propaganda for proper storage in the home of all cleaning fluids is the most important factor as the campaign for "protection seals" has been unsuccessful. The kitchen should be furnished with special cupboards which are out of reach of small children.

#### *S. P. Fällström: Jetex*

A  $2\frac{1}{2}$  year old boy was brought to the pediatric clinic of Gothenburg one hour after eating one or two "Jetex" tablets. On the way to the hospital he had general convulsions. On admission he was unconscious, hypertonic and had increased tendon reflexes. Respiration was shallow and slow, and respiratory arrest soon developed. He was treated symptomatically, and he breathed spontaneously after three hours. The next day he was completely asymptomatic. "Jetex" tablets are used for combustion in toy motors and contain about 0.75 g potassium bichromate. This, however, gives other symptoms of intoxication than those which the boy presented. The tablets also contain a combustible substance of unknown nature which probably caused the intoxication.

#### *B. Vahlquist: Pica of matches*

My case is rather unusual. A 14-year-old girl had been consuming match heads in increasing quantities over a period of several months—in the end several boxes a day. On admission to the Department of Pediatrics, Uppsala (record no. 184/60), she showed signs of grave anemia, with a hemoglobin level of 5.8 g per 100 ml, a red-cell count of 2.1 million, and a colour index of 0.9. Clearly, there was a combination of severe iron deficiency (serum iron 8 gamma%, bone-marrow iron extremely low) and potassium chlorate poisoning. After the patient had stopped eating match heads and had received iron therapy, the red-cell count rapidly rose and the hemoglobin level slowly improved, with the result that the colour index fell to 0.6, a value typical of simple iron-deficiency anemia. Six months after the discontinua-

tion of iron therapy, the symptoms of pica began to appear again. Examination disclosed recurrence of the anemia, this time with a picture of simple iron deficiency. A box of Swedish matches contains 0.3–0.5 g  $\text{KClO}_3$ , and in addition small quantities of  $\text{K}_2\text{CrO}_4$ ,  $\text{KMnO}_4$ , sulphur, and glue. Moeschlin gives the acute toxic dose of  $\text{KClO}_3$  for adults as 5–10 g. The daily consumption of up to 0.5 g or more for several months might well produce serious toxic effects. Iron-deficiency anaemia is a common cause of pica. It should always be excluded first in older children and adults, since other causes (apart from pregnancy!) are less common. In young children pica involving a variety of substances may almost be regarded as a normal phenomenon during a certain stage of development.

#### *R. Tunell and B. Persson: Chronic vitamin A intoxication in infants*

About 40 cases of chronic vitamin A intoxication in infants, children and adults are reported in the literature. The dosages have in general been high (150,000–500,000 IU/day) and the duration of overdosage long (at least  $\frac{1}{2}$  year with few exceptions). For these reasons a report is given of three infants, each of whom received vitamin A 22,000 IU/day during a period of 1–3 months with symptoms of vitamin A intoxication. *Symptoms and signs:* History of sudden onset of hyperirritability, tenderness and edema in the occipital region, signs of increased intracranial pressure (diastasis of the sutures, bulging fontanel, sunset sign) and a pronounced craniotabes were present in all three infants. All had an infection. Two of the infants had typical desquamation of the skin on the palms and soles. One infant had hypertonia. *Laboratory data:* Two cases had anemia, 2 cases had high values of alkaline phosphatase. All three infants had typical X-ray changes of rickets, none had cortical hyperostosis. All had normal liquor. Fasting vitamin A blood levels were obtained 3 days after vitamin A had been discontinued in one infant and 30 days after discontinuation in another. These values were 759 IU/100



ml and 410 IU/100 ml serum respectively. The upper limit of normal is 180 IU/100 ml. This examination was not performed in the third case. *Dose and duration:* All received 22,000 IU/day as 30 drops of "AD-vimin Astra", 1 month in one case and for 3 months in the other two. In 2 cases the dose had been prescribed by a physician for treatment of craniotabes, the overdosage in the third child was the result of carelessness by the mother. *Course:* All symptoms disappeared when the vitamins were discontinued.

*Comments:* The manifestations of vitamin A intoxication in this age-group are characteristic except for the X-ray evidence of rickets which has not been previously reported in man. However, rickets has repeatedly been induced in animal experiments and is said to depend on antagonism between vitamin A and D. The importance of care in prophylactic treatment of infants with water-soluble preparations of vitamin A is stressed.

#### Meeting Dec. 3, 1960

Guest lecture by *R. Debré, Paris:* Quelques études sur le sommeil de l'enfant et ses troubles

*Symposium: Swedish help to children in economically underdeveloped countries. Moder-*

ator: A. Wallgren. Participants: M. Levinson, S. Heppling, G. Herlitz, G. von Sydow and B. Strindberg. Discussion: J. Asplund and M. von Malmberg. (Will be published elsewhere.)

#### Meeting Jan. 13, 1961

*Jens Bergstedt:* Does Xantocid have any effect on the streptococcal flora in the throat?

During the spring of 1960 at Stockholm's Hospital for Infectious Diseases, a clinical bacteriological investigation was done to determine the effect of the preparation Xantocid on the  $\beta$ -hemolytic streptococcal flora in the throat. Xantocid is composed of Xantocillin and Thyrotricin, in the form of a lozenge, one administered every hour or every 2 hours. Our case material consisted of 38 children between the ages of 3-15 years who had been admitted to the scarlet fever ward. Bacterial cultures for streptococci as well as general flora were made from throat swabs taken on admission and every morning. Four to seven lozenges were given for 3-5 days.

Of 22 patients with uncomplicated scarlatina, 17 had hemolytic streptococci (++) on admission, while 21 had streptococci (++) after Xantocid therapy. Of 12 patients with scarlatina and impetigo or with lymphadenitis, 10 had streptococci (++) on admission and 9 after therapy. One patient

became bacteria free, but pharyngitis remained and he was subfebrile. Of 4 patients with the diagnosis desquamation of scarlet fever, scarlatina or streptococcal angina, 2 had hemolytic streptococci (++) on admission and 4 (++) after Xantocid therapy. The chances of finding  $\beta$ -hemolytic streptococci in the throat are greater in the morning, so that any substantial increase of the bacterial flora is not likely.

Of all 38 patients, 13 (one-third) had signs of infection remaining after Xantocid therapy. One developed otitis media and another streptococcal vulvovaginitis during this therapy.

This investigation shows that Xantocid does not appear to affect an abundant streptococcal flora in the throat. One cannot expect it to be an effective weapon against the spread of  $\beta$ -hemolytic streptococci in, for example, schools or closed institutions, as the manufacturer presumes. It is true that a scarlet fever ward is an environment rich in bacteria, but so is the classroom and the family milieu.

**B. Ursing: Immune-electrophoresis of the spinal fluid in cases of infections of the central nervous system and of polyradiculitis**

By means of immune-electrophoresis, the spinal fluid has been studied from 19 cases of parotitis meningitis, as well as 17 cases of viral meningitis of unestablished etiology, 5 cases of bacterial meningitis and 5 cases of polyradiculitis. This report concerns the occurrence of pronounced  $\alpha_2$ -macroglobulin,  $\beta_1$ -lipoprotein, fibrinogen, transferrin of the serum type,  $\beta_2$ M- and  $\beta_2$ A-globulin, as an expression of pathological protein patterns.

Two cases of parotitis meningitis and 4 of the cases of viral meningitis had normal spinal fluids. No case of bacterial meningitis or polyradiculitis had normal spinal proteins. Instead these showed the most pathological picture of all CNS diseases.  $\beta_2$ A-globulin was the commonest pathological fraction in parotitis meningitis and  $\beta_1$ -lipoprotein in the other viral meningitides. In bacterial meningitis and polyradiculitis, nearly all the above-named substances were present. Fibrinogen could be demonstrated in the spinal fluid when the protein level was 27 mg %. Transferrin of the serum type was unquestionably commonest in bacterial meningitis and polyradiculitis. The spinal fluid was most pathological at the peak of the disease, after which a relatively parallel restitution occurred. The pathologic fractions are not thought to have any importance in regard to the development of post-encephalitic symptoms. In bacterial meningitis and polyradiculitis, the spinal fluid changes sometimes persist after the patient has recovered.

**B. Thalme, I. Jungner and B. Åberg: The use of the ultramicromethod in a clinical chemical laboratory**

The analysis of chloride, calcium, phosphorus, urea and total proteins in blood serum has been carried out using Beckman/Spinco's modification of the ultra-micro technique of Sanz. The colorimetric methods can at present be entirely evaluated as the manufacturer has not been able to

deliver the "micromixer" part of the equipment. The titration methods give satisfactory accuracy for practical clinical work, but are not as precise as the corresponding macromethods. The authors would like to stress that it is necessary that the personnel who work with this technique be extremely well trained. The method's greatest advantages are that it saves space and glassware, and that material consumption is, above all, especially low. Therefore, this method ought to have a definite place in pediatric practice, as well as in work with experimental animals, rats, mice and so forth.

**Å. Espmark and E. Rabo: Smallpox vaccination during the first trimester of life: Percentage of positive reactions in relation to vaccine strength**

In *Svenska Läkartidn* 56: 82, 1959, Rabo has given an account of the percentage of positive reactions and advantages of vaccination during the first months of life. In a new investigation more than 300 children have been vaccinated, partly with undiluted vaccine and partly with vaccine dilutions down to 1/316. The "takes" with different vaccine titers vary from 2 to 100 %, and appear to follow a normal distribution curve. A group of children, 5-12 months of age, have been vaccinated with the same vaccine dilutions. In order to obtain the same percentage of positive reactions in both groups, one must use a vaccine ten times stronger for the younger children. This investigation has shown that one may obtain the desired percentage of "takes" even close to 100 %, by choosing the appropriate vaccine titer.

**Å. Espmark and E. Rabo: Antibody formation following smallpox vaccination before the age of 35 days**

Two groups of children, the first one month of age, and the second 9-12 months old, were investigated with regard to the presence of neutralizing antibodies before and after smallpox vaccination. Twenty-six children in each age group have until now been examined serologically. The titration

of antibodies was done by a screening method, whereby filter paper discs with serial dilutions of serum were placed directly in virus-infected tissue cultures. Localized viral growth inhibition was interpreted as neutralization.

Of the 26 younger infants, 23 had antibodies in the prevaccination serum. In the older group, antibodies were found in two of 26. In sera taken 1 month after vaccination, the titer values obtained ranged from 1:10 to 1:100, with a distribution which suggested a slightly lower average value for the younger group. The further development of these titers will be estimated by repeated titration of sera taken one year after vaccination. Until now 9 months' sera have been taken from five children in each age group and titrated simultaneously with the serum samples from the same infants obtained earlier. No obvious difference in the 9

month titers of the two groups was demonstrated. The number of samples is, however, too small for minor differences to be verified. One month after vaccination, six of the ten infants had titers 1:100 and four had titers 1:32. In the 9 month sera, four of the higher titers had decreased three-fold, while the lower titers remained unchanged.

The question as to whether the lower antibody formation demonstrated in the 1 month old infants depends on the maternal antibodies or on some incompleteness in the immunological maturity cannot as yet be answered by our results. In any event, the difference demonstrated is small, and it may be assumed that even in the younger infants, the first vaccination carries out its most important function, that is to produce the sensitization to vaccinia virus which conditions a good booster response to revaccination.

#### Meeting Feb. 10, 1961

##### *B. Persson and G. Sterky: A case of non-hemolytic hyper-bilirubinemia*

A 13 year old girl with persistent hyperbilirubinemia since birth is described. She had no family history of jaundice and was asymptomatic except for iron deficiency anemia at the time of her last examination. The level of bilirubin through the years varied between 3.6 and 10.0 mg % with negative direct and always strongly positive indirect Hijman van den Berghs reaction. It could be shown (eg. normal COHG and Cr<sup>51</sup> survival) that no hemolytic process was involved. Liver function tests, liver histology and cholecystography were normal. The case was diagnosed as Gilbert's disease. The authors discuss the progress made during the last few years in understanding defects in bilirubin metabolism. The difficulty of drawing conclusions from the results of loading with salicylate is underlined and the findings at chromatography of bile acids discussed. In this patient, a very low level of chenodeoxycholic acid was found which may suggest the presence of other enzymatic

defects in the liver cells. The existence of hitherto unknown pathways of bilirubin metabolism are considered.

##### *N. Engström and B. Persson: Hypoproteinemia with edema and albumin loss in the gastro-intestinal tract*

A preliminary report is given about an infant with polycystic kidneys, intestinal edema and hypoproteinemia who has been under observation since 4½ months of age. Serum-protein levels varied between 2.5-3.6 g %. Laboratory examination of blood and urine were normal. Liver, pancreas and adrenal function as well as intestinal absorption of fat and carbohydrates are normal. Studies with I<sup>131</sup> albumin i.v. revealed a loss of albumin into the gastrointestinal lumen estimated to be about 4 g per day. Treatment with high doses of albumin i.v. had only a transient effect. Triamcortone was of no value. A repeated I<sup>135</sup>-albumin study during a trial course of human growth hormone showed no change albumin loss, but suggested increased protein synthesis.

Hypoproteinemia in children is discussed. The albumin studies were performed together with a group at the Gustaf V Forskningsinstitut, Stockholm. The growth hormone was kindly put at our disposal by Prof. Gemzell, Uppsala.

*Yngve Larsson, Göran Sterky, Kristina Ekengren, and Tage Möller: Physical fitness and the influence of training in diabetic adolescent girls*

The physical working capacity (PWC) was studied in a group of 22 adolescent diabetic girls and compared with that of a control group of 27 non-diabetics of the same sex and age. The PWC was slightly lower in the diabetic group, the difference being most evident in the age-group of 15-18 years. In the diabetic group, the relations between PWC and body weight, age and heart volume were the same as among non-diabetics. There was a regular decrease of blood sugar during work and a highly significant correlation between the initial blood sugar value and the extent of blood sugar decrease. After training in a moderate long-term as well as in a strenuous short-term program, the PWC increased in the majority of the diabetic patients. It was most marked in the girls of 13-14 years who took part in hard training, a pronounced improvement being noticed also in the diabetic state, in spite of unchanged insulin dosage and a 50% larger caloric supply. There was a significant rise of blood cholesterol during the work tests. Also, the levels of cholesterol and triglycerides in the blood (but not of phospholipids) were significantly increased, when determined a few days after the severe training program.

The value of exercise and training in the treatment of juvenile diabetes is emphasized, the favourable effect being especially conspicuous in patients with a high blood-sugar level. During periods of intensified physical activity, the caloric supply should be increased, rather than the insulin dosage decreased.

*C. G. Bergstrand and M. Otto: Attempted suicide in children and adolescents*

The incidence of attempted suicide in persons 21 years of age or less was thought to be of interest. Four hundred and seventy-one hospitals in the entire country were requested to send in the appropriate records during the 5-year period 1955-1959. A total of 1727 cases (351 boys (20.3%) and 1376 girls (79.9%)) was collected from 458 institutions (97.3%). The youngest case was a 10½ year old boy. The incidence of suicide attempts increased with age and was greatest at puberty. An absolute increase in incidence was also noted during the 5 year period of study. Seasonal variation was also noted. The smallest number of cases occurred in June and July (92 and 81 cases respectively) and the largest number in November (147 cases). Thirty-eight per cent of the cases came from Stockholm, Gothenburg and Malmö, the inhabitants of which account for one-fifth of the total population of Sweden. The majority (82.5%) were from the lower class and 4.6% were foreigners (78 cases). Alcoholism was found in 14.6% and psychiatric disease in 27.6% of the parents. Forty-three per cent of the cases came from broken homes. Psychiatric disease was present in 19% of the boys and 8% of the girls. The method of suicide used by boys tended to be more active (hanging, shooting), while girls tended to take overdoses of tablets, etc. Twenty-nine per cent had attended a psychiatric clinic or been in mental hospitals while 70.4% had been treated for somatic disease. Psychiatric consultation was obtained in 69.4%. A need for continued psychiatric care was found in 31% of the boys and 19.3% of the girls. Fifteen per cent of the persons had previously attempted suicide, and this was more common amongst the boys.

*K. M. Herrlin and P. O. Hillborg: Cerebral symptoms in the juvenile form Gaucher's disease*

Six familial cases of Gaucher's disease are described. The patients are 6-20 years old and are all alive. Diagnosis has been con-

firmed in two by chemical analysis of the spleen. Splenectomy was performed at 1½–12 years. Within 3 years of the operation, skeletal changes occurred in all cases. Cerebral symptoms have gradually also appeared in all after operation. Five are mentally retarded. Muscular incoordination primarily affecting the eyes is present in 5, while 3 also have trismus. Three have epilepsy. All of the patients have abnormal electroencephalograms, 3 of which show significant changes with spikes, and sharp and slow waves with shifting localization. The cerebral symptoms are similar to those found in the infantile form in which they are attributed to deposit of cerebrosides in the reticuloendothelial cells of the brain vessels. The pathogenesis in the juvenile form is probably the same. It is possible that the cerebral symptoms as well as the early appearance of the skeletal changes have been precipitated by splenectomy. (Will be published in *Acta Paediat.*)

*C. G. Bergstrand, Birgit Czar and P. H. Tarukoski: Serum haptoglobin in infancy*

Haptoglobin is defined as group of mucoproteins with the electrophoretic properties of  $\alpha_2$ -globulins. Haptoglobin forms with hemoglobin a very stable complex with peroxidase activity which may, according to Jayle, be used for quantitative determination of the serum haptoglobin content. With Jayle's activation method, the haptoglobin level in sera from fetuses, newborns and infants was determined. In fetal and cord blood, no haptoglobin was demonstrated. During the neonatal period (full term and premature infants), small amounts of haptoglobin were found only in a few cases. After the newborn period, the haptoglobin level tends to rise gradually and after 5–6 months of age, values below 30 mg per 100 ml serum, i.e. the lower limit for normal adults, are rarely demonstrated. Fetal hemoglobin bound to haptoglobin seems to have a peroxidase activity which is higher than the corresponding complex of adult hemoglobin. Preliminary experiments suggest that haptoglobin injected intravenously into newborn infants is rapidly eliminated.

## ANNOUNCEMENT

### The X International Congress of Pediatrics

The X International Congress of Pediatrics will be held in Lisbon, Portugal Sept. 9 to 15, 1962 with Professor C. Salazar de Sousa as President and Professor Marie Cordeiro as General Secretary. There will be three plenary sessions, which will take up three mornings with the following items: 1. Problems of the newborn infant.—2. Pediatric problems of tropical climates and coun-

tries in development.—3. Pediatric doctrine. The remaining scientific programme is divided into 15 sections, for each of which subjects of more topical importance has been chosen. An extensive social program is planned as are scientific exhibits, industrial exhibits, and exhibitions of art and children's literature. The address of the Secretariat: Av. 28 de Maio, Lisbon, Portugal.

## ANNOUNCEMENTS

### Congresses

#### The II International Congress of Mental Retardation

Vienna, August 14-19, 1961. President: Professor K. Kundratitz, Pediatric Clinic, Lazarettsgasse 14, Vienna, Austria. The principal subjects to be discussed are: 1. Organic causes of mental retardation.—2. Endocrine disorders and mental retardation.—3. Encephalopathy and mental retardation.—4. Genetic and metabolic problems.—5. Mental retardation caused by psychosis in childhood.—6. Pseudo-feeble-mindedness.—7. Diagnostics and tests.—8. Evaluation of histories.—9. Social adjustment of mentally retarded children.—10. Child guidance.—11. The physician and special schools.—12. Pharmacotherapy.

#### The Annual Congress of the Pediatric Society of North-Western Germany

Lübeck, June 2-4, 1961. President: Professor J. Jochims, Kinderklinik, Kronsförder Allee 71/73, Lübeck, Germany. There will be refresher courses in Orthopedics and in Dermatology. The scientific subjects to be discussed are: 1. Constitution and 2. Psychotherapy.

#### The 60th Congress of the German Pediatric Society

Heidelberg, Sept. 11-13, 1961. President: Professor Ph. Bamberger, Universitäts-Kinderklinik, Heidelberg, Germany. The principal subjects are: 1. Convulsions in childhood.—2. Inborn errors of metabolism.—3. Malignant tumors in children. Information may be obtained from the General Secretary, Professor K. Schreier, Universitäts-Kinderklinik, Heidelberg.

#### The XIII Congress of the Nordic Pediatric Association

Copenhagen, June 25-29, 1961. President Dr. med. P. W. Braestrup, General secretary Dr. med. E. Winge Flensburg. Principal subjects to be discussed: 1. The influence of prenatal and natal factors for development and for diseases; prespective and longitudinal studies.—2. Epilepsy in childhood.—3. Steroid treatment of acute life-endangering infections.—4. Prognosis of asthma and asthmatic bronchitis in children. Address of the Secretariat, Amtssygehuset, Hellerup, Denmark.

## BOOK REVIEWS

*André-Thomas, Yves Chesni and S. Saint-Anne Dargassies: The Neurological Examination of the Infant.*

National Spastics Society, London, 1960. 50 pages. Price 5/- net or \$1:—.

This is the first number of "Little Club Clinics in Developmental Medicine" published as Supplements to the excellent Cerebral Palsy Bulletin. It gives an account of some guide lines of neurological examination and some typical responses of the

normal newborn and older infants. The aim is to make a topographical diagnosis and to describe the neurological mechanisms implicated. The editors of the booklet, R. C. MacKeith, P. E. Polani and E. Clayton-Jones, give introductory information concerning the type of examination used by André-Thomas as well as an interpretation of the findings. The text is richly illustrated by drawings from photographs and ciné films. This is a useful booklet for pediatricians and students.



*L. Emmet Holt, Jr., P. György, E. L. Pratt, S. E. Snyderman and W. M. Wallace: Protein and Amino Acid Requirements in Early Life.*

New York University Press, New York, 1960. 63 pages. Price \$1.00.

In this pamphlet present knowledge of the protein and amino acid requirements in early life are summarized. The following items are discussed: protein intake and body composition, evaluation of protein requirements and of protein quality, amino acid requirements and amino acid balance, desirable intake of protein and amino acids and the need for further research. It is not possible to go into details. Be it sufficient to say that the responsible authors, who are well-known experts in pediatric nutrition, have critically evaluated the current literature. About 200 references.

*E. Gedda: The Significance of the Premature Child's Low Birth Weight for its Vitality and Social Prognosis.*

Almqvist & Wiksell, Uppsala 1960. Thesis, University of Gothenburg.

This work has special value as a parallel and complement to a study made by Ingvar Alm entitled "The Long-term Prognosis of Prematurely Born Children" (*Acta Paediatr*, Suppl. 94, 1953). Like Alm's work it is based on material culled from maternity hospitals, well-baby clinics, school health services and official social registers, such as that of the Royal Temperance Board and Central Register of Criminology. Social status and adaptation as well as income were also studied. Alm's material consisted of 999 prematures and 1002 full-term babies (as controls) born 1901-1921 in three maternity hospitals in Stockholm. The present material consists of 742 prematures and 426 controls born 1930-1936 in the maternity hospital of Gothenburg. Alm's material was selected, and only included boys legitimately born. Gedda's material comprises all prema-

tures, girls as well as boys, regardless of legitimacy and also stillborns of the same birth weight. Gedda's material is probably more typical of the population of Gothenburg than Alm's was of Stockholm, as the percentage of institution deliveries was higher in Gothenburg in the thirties (82-85%) than in Stockholm during the early decades of this century (50-75%).

Gedda has followed the same principles as Alm when subgrouping the material by dividing the children into single and multiple births. The tables are arranged in the same way as Alm's, which makes comparison easy.

Factors which may influence birth weight are considered. As this material is based on obstetrical case histories which are 25-30 years old, the results are somewhat difficult to evaluate. Gedda then examines the mortality rate at different ages and has been able to follow nearly all living patients to the age of twenty. The following results were the most important.

During the period 1930-1936, prematures constituted about 5% of all live births in Gothenburg. Their mortality during the first week of life was 19.1% as compared with 0.7% for children born at term and their perinatal mortality 30.2% as compared with 2.1% for controls. The infant mortality minus the mortality during the first week was 7.2% for prematures compared with 1.1% for controls. During the second year of life, the mortality was 3.1% for prematures and 2.4% for controls. After five years of age no difference was found in the mortality risk between the two groups. The difference in morbidity declined similarly and was not present at school age, apart from that associated with sequelae from birth injuries, which are probably more common among prematures. Prematurely born children were considered by physicians as well as by their parents as "fragile" more often than were the controls. This may be due more to their attitude than to prematurity *per se*. It was shown that premature children on the average were somewhat smaller, even at school age. Regarding psychic develop-

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ment, the investigation could not verify any significant difference between prematures and controls, but here a more detailed study seems indicated. Like Alm, the author found no difference between prematures and controls regarding social capacity, working capacity and social adaptation. Though the author's figures indicate an increase frequency of prematures amongst retarded and other minus variants in school-age, he has furnished evidence that the premature child's handicap has been principally overcome by the age of one year.

**P. Végelyi. Die Künstliche Hibernation.**

Hungarian Academy of Sciences. Budapest. 1960. 612 pages, 107 figures. Price not given.

The well-known Hungarian pediatrician, Végelyi, has written this important monograph on the theoretical problems and practical experiences of artificial hypothermia both chemically and physically induced. Every aspect of the problem is considered. The indication, technique and clinical experience in various fields of medicine as well as the contra-indications and side effects of hibernation are described in detail in separate chapters. For a pediatrician the experiences of induced hypothermia in the treatment of perinatal disorders, infantile diarrhoea, infectious diseases and Waterhouse-Friderichsen syndrome are of special interest. Due consideration is paid to the publications and experiences of other investigators and the tabulated references number 1568.

**F. G. Young (Editor) et al. Insulin.** British Medical Bulletin. Volume 16, Number 3, September 1960, 167-264. Price 20 Sh.

This publication represents a most valuable and competent review on insulin by leading Canadian and British authorities. Forty years have passed since the discovery of the hormone, and the activities of the pioneer Toronto institute during this eventful period of medical history are described by Charles H. Best in the introductory paper. Next, F. Sanger in an article on the chemistry

of insulin shows how through the use of advanced chemical technique he succeeded in finding the double-chained structure of the molecule. The problems of insulin assay both *in vitro* and *in vivo* are analysed in several papers and comparison made between the often remarkably divergent results obtained with different methods. Insulin antagonists and insulin antibodies are also reviewed and as regards the labile type of juvenile diabetes the hypothesis is given (Vallance-Owen) that fluctuating overaction of the insulin-antagonistic pituitary-adrenal system may be a cause of the "brittleness", and that treatment with adrenal steroids in doses sufficient to suppress the endogenous production of these hormones might stabilize the disease. The mechanism of action of insulin is a field of some controversy illustrated in several contributions. Some investigators believe that the manifold actions of insulin with effects on carbohydrate (glycolysis), fat (lipogenesis) and protein (growth) metabolism are explicable in terms of a single effect, probably located to the processes governing the entry of glucose into the cell. Others believe that insulin acts on different intracellular metabolic pathways or enzymatic (hexokinase) reactions. "Nevertheless, the glimmerings of a reasoned unity are beginning to be discernible in the fog of uncertainty", (Randle & Young). The last part of the volume has a more clinical aspect, discussing indications and contraindications for the use of different insulin preparations, treatment of ketosis, reactions, etc. Finally two articles describe the chemistry and clinical use of hypoglycaemic substances other than insulin, the sulphanilyl as well as the guanidine derivatives. In juvenile diabetes the effect of the former is only temporary during an early phase of the disease, but in adults, too, the incidence of secondary failures is increasing. As regards the guanidine preparations their mode of action as well as indications for use are still unsettled. Until more is known it seems wise to use them only in carefully conducted clinical trials.

Yngve A. A. Larsson, Stockholm

*Stanbury, Wyngaarden and Fredrickson. The Metabolic Basis for Inherited Disease. McGraw Hill House, London, 1960, 1477 pp. Price £ 11 12 s.*

The state of current knowledge and thought regarding the biochemical and genetic bases of inherited disease is comprehensively presented in this volume. To accomplish this task the editors have called on 46 contributors, including themselves. The book is divided into ten sections, each of which is concerned with the disturbances primarily involving a particular system. The grouping of diseases with disturbances in the same system permits the use of general introductory remarks concerning normal biochemical pathways. It also encourages continuity of thought.

Although primary consideration is given to the description of normal and disturbed biochemical processes and to the genetics involved, brief clinical and therapeutic descriptions are also included. To assist the reader the editors have apparently insisted that frequent summaries and recapitulations be inserted in each chapter. A bibliography, which is extraordinarily current (with many references from 1960), follows each chapter.

A great deal of time has obviously been spent in compiling the index. It is 52 pages long and as best as the reviewer can discover by random testing, every subject has been included with the additional indication of whether the reference is a minor or main discussion of the subject. Another helpful feature is an appendix which describes the methods of coding and filing family records.

All of the chapters are well written; even so, the reviewer wishes to call attention to the first chapter in which the editors have collaborated. Here the concepts of inherited disease are reviewed and exemplified with a clarity and style that is a joy to read.

This volume is enthusiastically recommended for medical school and hospital libraries, where it is a must, as well as for all individuals more than remotely interested in inherited diseases.

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*Edited by F. Linneweh. Die Prognose chronischer Erkrankungen. Long-term observations of chronic diseases. Springer Verlag, Berlin, Göttingen, Heidelberg 1960. 404 pages. Price 98 DM.*

In the summer of 1959 a European conference on the long-term prognosis of chronic diseases was held at Herrenchiemsee, Germany. The organiser, F. Linneweh, edited this summary of the proceedings in English or German. About fifty specialists in pediatric and adult general medicine present many new data in this hitherto neglected field. Much valuable information is given on the long-term prognosis of most chronic pediatric diseases and also on how children live with their disease. Only some main features will be mentioned here. Pylonephritis has a worse prognosis than glomerulonephritis. Intensive and prolonged chemotherapy is recommended in trying to improve the results. The prognosis of juvenile obesity is very poor and longterm psychotherapy seems indicated. H. Asperger found adults who had had enuresis to be neurotic and infantile with poor social adjustment. This study and that on ventricular ulcer (by A. Jores) requires control studies and stricter definitions of the psychiatric pathology found. Linneweh presents a series of premature infants with hyperbilirubinemia treated by exchange transfusions on clinical indications alone (mostly based on neurological signs). Sixteen patients without clinical signs of bilirubin-encephalopathy whose serum-bilirubin rose to 21–32 mg% were not given exchange transfusions, and were found normal at the age of 1½–3½ years. The severe prognosis in juvenile asthma is stressed, but no information on the long-term effect on the respiratory function are given. Conspicuously good long-term results in the treatment of cystic fibrosis of the pancreas are reported from Queen Elizabeth Hospital for Children, in London.

Clinicians can learn much from this book. Many of the reported results are a challenge to an intensified search for better therapy.

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## Undergraduate Paediatric Education in South-East Asia

by P. ROBINSON<sup>1</sup>

### Introduction

The discipline of paediatrics appears to be least developed where it is most needed, and the number of paediatricians seems smallest where problems of child health are most pressing. This unfortunate situation is further aggravated by the fact that the smaller the number of paediatricians in a country, the less trained in paediatrics are the general practitioners.

Studies of paediatric education have been conducted in the U.S.A. (1), Canada (2), Europe (3) and Latin America (4). The present paper describes the situation in the countries of the South-East Asia Region of W.H.O. as of the end of 1959.

### 1. Sources of Information

Some data on paediatric education in India are contained in a review of Indian paediatrics in 1955 (5), brought up to date in 1958 (6). A few details on other countries of the region are contained in a series of articles on maternal and child health services in South-East Asia (7, 8, 9).

At a seminar on paediatric education

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<sup>2</sup> In Burma, Ceylon and India the term "medical college" is used to depict a full-fledged medical training institution of university standard.

in South-East Asia, held under W.H.O. auspices in Bangkok in June, 1958, eighteen paediatric teachers discussed the subject, and provided a considerable amount of information, some of which is used in this paper. A final report on this seminar is, unfortunately, not yet available.

A questionnaire was distributed in 1959 by the W.H.O. Regional Office for South-East Asia to all medical schools<sup>2</sup> in the region.

Finally, the present writer has visited at least once, often a few times, 34 of the 46 institutions in which paediatrics is being taught at present.

### 2. General Remarks on Medical Education in S.-E. Asia

In *Afghanistan*, medical students pay no tuition fees and receive free accommodation, food, clothing and pocket money. There is an internship year, out of which two months are spent in the paediatric department. After graduation (Dr. med.) the doctor becomes automatically a government servant.

In *Burma*, there were no tuition fees till the beginning of the academic year 1959-60. From now on, students pay the equivalent of three dollars per month.

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TABLE 1. *Medical schools in S.-E. Asia.*

Country	Duration of medical course in years	Internship	Degree	Medium of instruction	Tuition fees	Service to government
Afghanistan	6	Compulsory	Dr. med.	Farsi	Nil	Permanent
Burma	5 <sup>a</sup>	Not available	M. B., B. S.	English	\$3 per month	Nil
Ceylon	5 <sup>b</sup>	Available, not strictly enforced	M. B., B. S.	English	Nil	Nil
India	5 <sup>a</sup>	Compulsory, where available	M. B., B. S.	English	Average \$50 per year	Nil
Indonesia	7 <sup>c</sup>	Part of the curriculum; 2 years	Dr. med.	Indonesian	\$5 per year	Three years after graduation
Portuguese India	5½	6 months; compulsory	Médico-Cirurgião	Portuguese	Nil	Nil
Thailand	4	Available, but not compulsory	M. B.	Thai (with technical terms in English)	In stage of revision	One year for each year of subsidized studies

*a* Plus two years' pre-medical training.

*b* Plus one year preparatory course.

*c* Being reduced now to six years.

There is no internship year. The qualification is M.B. (Bachelor of Medicine), B.S. (Bachelor of Surgery).

There are no tuition fees in *Ceylon* either, but students who fail in examinations and have to repeat a course, pay the equivalent of \$ 35 per semester. Full registration is granted after one year's internship in an approved hospital. Provisional registration is permitted immediately after graduation. The qualification is M.B., B.S.

In *India*, annual tuition fees range from the equivalent of \$25 (in six institutions) to \$100 (in one only), the average being \$50. Recently, an internship year has been introduced in some, or most, of the medical colleges, which is now in the process of being reduced to six months. There is no definite provision for part of the

internship service to be spent in paediatrics. The qualification is M.B., B.S.

In *Indonesia*, the annual tuition fees are 240 rupiahs (about \$5 at the present official rate of exchange). There is no internship, but during the sixth year of studies, the student does practical work in the paediatric department. Graduates must serve the government for three years. The qualification is Dr. med.

In *Portuguese India* there are no tuition fees. After graduation there is a six-month compulsory internship. Paediatrics for this purpose is part of the obstetric training. The qualification is "Médico-Cirurgião", and is not recognized outside the colony.

In *Thailand*, there were no tuition fees till recently. Now, students can only be exempted if they undertake to serve the

TABLE 2. *Students in paediatrics in S.-E. Asia.*

Country	Total number of medical teaching institutions	Medical schools teaching paediatrics						
		No. of institutions	No. of students	No. of students admitted 1959		No. appeared for examination 1959	No. passed	% failure
				M	F			
Afghanistan	1	1	355	83	27	33	33	0
Burma	2	2	1,381	229	100	238	104	55
Ceylon	1	1	699	108	27	156	123	20
India <sup>a</sup>	52	36	18,517	2371	967	4979	2603	48
Indonesia <sup>b</sup>	6	4	4,068 <sup>c</sup>	488	73	253	205	18
Portuguese India	1	1	100	23	Nil	Unknown	23	Unknown
Thailand	2	2	1,001	157	53	197	162	18

<sup>a</sup> Only 33 of the medical colleges teaching paediatrics graduated students in 1959.

<sup>b</sup> Only three of the medical faculties teaching paediatrics graduated students in 1959.

<sup>c</sup> This number includes students of dentistry in Jogjakarta.

government after graduation. Internship is not compulsory. The qualification is M.B.

The medium of instruction in *Burma*, *Ceylon* and *India* is English. In *Afghanistan*, national teachers teach in Farsi, and lectures of foreign teachers are being translated. In *Indonesia*, the medium of instruction is Indonesian, but guest teachers often use their own languages. In *Thailand*, while Thai is used, technical terms are in English. In *Portuguese India*, the medium of instruction is Portuguese.

### 3. Distribution of Schools and Students in Paediatrics

In one country of the region—*Nepal*—there is no medical school yet. In *Afghanistan*, the present output of graduates is considered sufficient for the time being. In *Burma*, the recently established second medical college in Mandalay is part of the University of Rangoon. In *Ceylon*, plans for the establishment of a second medical

college in Kandy are nearing completion. In *India*, 14 new medical colleges were established during the last four years making a total of 52. Only two of those have reached the stage of clinical teaching; four other medical colleges have not yet fully developed the clinical curriculum. Thus, paediatrics is taught at present in 36 out of the existing 52 institutions. In *Indonesia*, two medical faculties were established since 1955, making a total of six. Paediatrics is not taught yet in the new institutions. The "medical surgical" school in *Goa* (Portuguese India) is without full university status. In *Thailand*, in addition to the two medical schools in Bangkok, a third is under preparation in Chiangmai.

### 4. Paediatrics as a Speciality

In *Afghanistan*, where the faculty of medicine was established in 1932, there was no paediatric teaching till 1943. At present, paediatrics is a full-fledged de-

TABLE 3 a. *Departments of paediatrics: independent and dependent.*

Country	No. of institutions teaching paediatrics	Fully independent paediatric department	Not fully independent paediatric department
Afghanistan	1	1	—
Burma	1	—	1
Ceylon	1	1	—
India	36	26	10
Indonesia	4	1	3
Portuguese India	1	—	1
Thailand	2	2	—

partment, and is a "major subject" in the final examinations.

In *Burma*, the paediatric wards and out-patients' services are under a paediatrician, but administratively paediatrics is part of the department of medicine, and does not constitute a separate subject in the final examination.

In *Ceylon*, where the medical college was established in 1870, a chair of paediatrics has existed since 1949. At the final examinations paediatrics is included in medicine.

In *India*, all stages of transition from a "subspeciality of medicine" to a separate chair are found. However, in no institu-

tion is paediatrics considered a "major" subject, nor is it a subject for the final examinations; usually one or two questions in the medical examination paper deal with paediatric problems.

In *Indonesia*, the paediatric department of the faculty of medicine of Djakarta is a large unit under a full professor. Paediatrics is considered there a "major" subject, and a special examination is held. In the other medical faculties, the paediatric units are small, and teaching is limited; administratively they are under the department of medicine.

In *Portuguese India*, paediatrics is not taught as a separate subject.

TABLE 3 b. *Paediatric departments in India: development during the last four years.*

Total number of medical colleges teaching paediatrics		Independent departments				Under the professor of medicine			
		In charge of full professors of paediatrics		In charge of an associate professor, a reader, a lecturer, or an honorary paediatrician		Paediatric department supervised by professor of medicine		Paediatric wards actually part of the medicine department, and all teaching done by the professor of medicine	
1955	1959	1955	1959	1955	1959	1955	1959	1955	1959
31	36	2	11	9	14	11	6	9	5



In *Thailand*, paediatrics was separated from medicine in 1945. It is not yet considered a "major" subject.

### 5. Teachers in Paediatrics

Some of the paediatric teachers are full-time, some are part-time, and some are honorary. With very few exceptions, however, the time devoted to teaching is independent of the status of the teacher. For the purpose of a general review it may be accepted that the actual time the chief of the department devotes to teaching is 1-2 hours a day in all, or most, institutions.

At Kabul medical faculty—*Afghanistan*—there is one professor, two associate professors, one reader and one lecturer. The ratio of teachers to students is one to seven.

In *Burma*, the teacher-student ratio is the lowest in the region, there being only one part-time lecturer for 240 students in paediatrics.

In *Ceylon*, there is one professor and one lecturer, but most of the teaching is done by junior assistants, after two years of internship.

In *India*, there are considerable differences. The highest number of paediatric teachers—in two institutions only—is six; the ratio of teachers to students in these two units is 1:20 and 1:35 respectively. The lowest ratio is 1:305. In five colleges there are as yet no special teachers in paediatrics and the subject is taught by the professors of medicine (see Table 3b).

In *Djakarta, Indonesia*, there is the largest number of paediatric teachers of any institution in the region—18. Fourteen of these are classified as lecturers. How-

TABLE 4. *Teacher-student ratio.*

Country	Average	Range
Afghanistan	1 : 7	—
Burma	1 : 240	—
Ceylon	1 : 45	—
India	1 : 70	1 : 300-1 : 20
Indonesia	1 : 18	1 : 100-1 : 9
Thailand	1 : 13	—

ever, the teacher-student ratio is only 1:9, lower than in Kabul. In the other medical faculties in that country there are very few paediatric teachers.

In *Portuguese India*, there are no separate teachers in paediatrics.

In *Thailand*, the teacher-student ratio is next only to Afghanistan, in this region.

### 6. Teaching Facilities

#### A. Hospital Beds

Traditionally, hospital beds are often considered the most important, sometimes the only, necessary facility for teaching of paediatrics. This is, of course, even less correct in this part of the world than in economically more advanced countries. Only very sick children can be admitted to paediatric departments of teaching hospitals here, and the student, if this is his only, or main, training field, sees chiefly the final stages of fatal conditions.

But even so, the numbers of beds in teaching hospitals are very small, and some of the so-called "paediatric beds" are for "surgical" conditions in children—actually under the supervision of the surgeons. Occasionally, the paediatricians may be called upon to advise on the general care of children hospitalized for surgical conditions, but as a rule, the surgical



TABLE 5 a. *Beds for children in teaching hospitals.*

Country	No. of institutions teaching paediatrics	Number of "medical" beds for children			No. of "surgical" beds for children		
		Infectious included	Infectious excluded	Separate infectious	Under surgeons	Under paediatric surgeons	Under paediatricians
Afghanistan	1	40	—	—	—	—	—
Burma	2	80	—	6	30	—	—
Ceylon	1	—	250	24	108	—	—
India	36	566	1096	94	635	65	10
Indonesia	4	380	65	20	—	—	—
Portuguese India	1	—	—	—	—	—	—
Thailand	2	135	60	80	12	—	—

paediatric beds are unrelated to paediatric teaching activities. In one unit only, in India, the surgical beds are under the care of the paediatrician, and in two other, also in India, under paediatric surgeons.

As a rule, "medical" and "infectious" beds are available for paediatric teaching purposes, but not necessarily all beds. Some institutions have special wards earmarked for teaching, and there is, in that case, administrative division between the service and the teaching unit.

#### B. Out-patient Services

Separate daily out-patient clinics for children are held in all but three of the teaching hospitals in which paediatrics is

taught. The average number of child out-patients per day ranges from 140 in Indonesia to 1200 in Ceylon. About 50 % are, as a rule, new cases. Very few, if any, nurses are available to assist the doctors.

Some of the paediatric teachers select a limited number of child out-patients for teaching, or have special sessions to which selected cases are invited. Although there appears to be no third alternative, these methods are unsatisfactory in as much as teaching is not done under normal conditions of service.

#### C. Preventive Child Care

Only five teaching institutions, all in India, take advantage of existing maternal and child health centres or child welfare centres for teaching of preventive child care. Preventive service units for children are available in all cities or towns where medical schools are located, but for various reasons those are not used for teaching. These reasons may be summarized as follows:

1. Insufficient awareness on the part of

TABLE 5 b. *Bed-student ratio.*

Country	Average	Range
Afghanistan	1:1	—
Burma	1:3	—
Ceylon	1:2	—
India	1:3	1:18-1:1
Indonesia	2:1	1:2-3:1
Thailand	3:2	—

TABLE 6. *Facilities for out-patient teaching.*

Country	No. of institutions teaching paediatrics	Out-patient services available for teaching	Average number of out-patients per day	
			Total	New
Afghanistan	1	1	200	60
Burma	2	2	230	110
Ceylon	1	1	1200	600
India	36	35	170	70
Indonesia	4	3	140	60
Portuguese India	1	—	—	—
Thailand	2	2	145	50

teachers of the importance of preventive paediatrics.

2. No teaching time allotted by the faculty for this purpose.

3. Difficulties arising out of the fact that the preventive service units are under a different authority from the teaching institution.

4. Some public health officials in charge of preventive services do not welcome teaching paediatricians to use their institutions.

5. Some teaching paediatricians prefer to have their own child welfare clinics rather than use existing ones.

In *India*, a number of teaching in-

stitutions now have departments of preventive and social medicine. Some of the professors of preventive and social medicine consider preventive paediatrics to be their domain. Where the teaching paediatricians are not too keen on preventive aspects, they welcome the initiative of the department of preventive and social medicine to take it on. In a few instances, the two professors co-operate in the teaching of preventive paediatrics, and again, in some others, the professor of preventive and social medicine is quite ready to give up some of the hours allotted to him to the paediatrician, for him to use it for teaching preventive paediatrics.

TABLE 7. *Facilities for teaching preventive paediatrics.*

Country	No. of institutions teaching paediatrics	Child welfare clinics available for teaching purposes	
		Attached to teaching hospital	Under different authority
Afghanistan	1	—	—
Burma	2	—	—
Ceylon	1	1	—
India	36	11	5
Indonesia	4	1	—
Portuguese India	1	—	—
Thailand	2	2	—

TABLE 8. "Rooming-in."

Country	Total number of teaching institutions with obstetric services	Infants with mothers	Separate nurseries
Afghanistan	1	1	—
Burma	2	—	2
Ceylon	1	1	—
India	35	30	5
Indonesia	4	—	4
Thailand	2	—	2

## D. Newborn Care

Newborn care is taught by obstetricians, paediatricians, or not at all. While some obstetricians consider newborns to be their domain, both in respect of teaching and service, others are only too glad to hand over supervision to the paediatrician.

The paediatricians, on the other hand, are often reluctant to assume responsibility for newborns because of lack of time.

An important reason for leaving care of, and teaching on, newborns to the obstetrician in some countries of this region is the prevalent system of "rooming-in". Where infants are with their mothers, it is simpler, and appears more sensible, that

whoever looks after the mother is also responsible for the infant.

"Rooming-in" is not an accepted principle in South-East Asia and, so far, no institution which had a separate nursery for newborn has changed back to "rooming-in". On the other hand, recent attitudes in western countries may have influenced institutions, at least in India, not to establish separate nurseries where such did not already exist.

## E. Special Clinics

There are a number of special clinics in India attached to some institutions, which are sometimes, but not invariably, used for undergraduate teaching. None in other countries, so far.

## 7. Hours and Methods of Instruction

Paediatrics is taught in the third, fourth, or fifth year, during one, two or three years. Experimentally, some aspects of preventive paediatrics are taught in two institutions in India also in the pre-clinical period, in co-operation with the department of preventive and social medicine.

TABLE 9. Newborn care.

Country	Total no. of teaching institutions with obstetric services	Newborns under paediatrician	Newborns under obstetrician			Premature unit	
			Paediatrician consulted regularly	Paediatrician consulted occasionally	Paediatrician consulted exceptionally	Under paediatrician	Under obstetrician
Afghanistan	1	—	1	—	—	—	—
Burma	2	—	—	—	2	—	—
Ceylon	1	1	—	—	—	1	—
India	35	4	7	20	4	3	4
Indonesia	3	1	2	—	—	1	—
Thailand	2	2	—	—	—	2	—

TABLE 10. *Special clinics.*

Child guidance	Handi-capped children	Neuro-logical	Nutrition	Spastics	Liver con-ditions	Tuber-culosis	Mental deficiency	Rheumatic heart disease
9	5	3	3	3	1	3	1	1

Didactic lectures range from a few, in some institutions in India, to 230 in Afghanistan.

Bedside teaching is practised in all countries, but in some instances this is actually didactic lectures delivered at the bedside, instead of in the lecture hall. Where sufficient junior tutors are available, bedside teaching is practised more efficiently.

Not much time is devoted to out-patients and child welfare teaching, for reasons already stated (see Section 5, paras. B and C).

There is no clerkship in Afghanistan, Burma, Ceylon, Portuguese India or in six of the teaching institutions in India. Clerkship from 2 to 107 weeks is available in all but six institutions in India and in

Indonesia and Thailand. Not infrequently, clerkship means little more than writing case sheets for the doctors.

Home visiting by students of paediatrics has recently been introduced in Colombo. In some of the Indian institutions home visiting is an important part of the activities of the department of preventive and social medicine, and some of it is concerned with child care. This is especially so, where the teachers of paediatrics and preventive and social medicine co-operate.

A major opportunity to impart techniques of health education is given in a large number of teaching institutions in India and Kabul through the presence of mothers on the wards. So far, little advantage is taken of this opportunity, although more

TABLE 11. *Hours of teaching.*

Country	Year(s)	No. of didactic lectures		Hours of bedside teaching		Hours of out-patient teaching		Preven-tive child care	No. of weeks of clerkship	
		Average	Range	Average	Range	Average	Range		Average	Range
Afghanistan	IV, V	230	—	85	—	85	—	Nil	Nil	—
Burma	V	20	—	12	—	12	—	—	4	—
Ceylon	III, IV	46	—	132	—	16	—	Yes	10	—
India	Variable	30	4-75	55	0-156	36	0-48	In 10 only	5	0-8
Indonesia	IV, V	108	12-200	43	25-79	37	12-60	Yes	7	2-14
Thailand	III, IV	75	73-77	75	60-90	42	24-60	In one school only	56	6-107

TABLE 12. *Attitude to mothers of hospitalized children.*

Country	Total no. of teaching institutions	Banned	On sufferance	Welcome
Afghanistan	1	—	—	1
Burma	2	2	—	—
Ceylon	1	1	—	—
India	36	4	14	18
Indonesia	4	4	—	—

and more institutions begin to realize the great possibilities of educating mothers during their stay in hospitals; this also permits medical students to study mother-child relationships and techniques of health education. The best facilities for mothers exist in Kabul, where they are given beds next to their children and food. In India, attitudes to the presence of mothers differ, and facilities for their accommodation are limited.

### 8. Trends and Outlook for the Future

The importance of teaching paediatrics as a separate discipline is growing in all countries of the region. Serious efforts are being made in this direction.

At the same time, more emphasis is being laid on teaching of promotional and preventive aspects of child care, and the use of peripheral services for instruction of medical students.

### References

1. American Academy of Pediatrics: Child Health Services and Pediatric Education. New York Commonwealth Fund 1949.
2. Canadian Society for the Study of Diseases of Children: Survey Canadian Medical Schools 1947-48. Toronto, privately printed, June 1949.
3. VEENEKLAAS, G. M. H.: Paediatric education in Europe. *Helv Paediat Acta*, 12: Suppl. 7, 1957.
4. WEGMAN, M. E., HUGHES, J. G. and PUFFER, R. R.: Pediatric education in Latin America. *Pediatrics*, 22: 205, 1958.
5. ROBINSON, P.: Pediatrics in India in 1955. *Pediatrics*, 19: 317, 1957.
6. — Undergraduate pediatric education in India. *Indian J Pediat*, 25: 283, 1958.
7. — Maternal and child health services in S.-E. Asia, II, III, IV. (a) Afghanistan, *J Trop Pediat*, 2: 18, 1956; (b) Burma, *J Trop Pediat*, 2: 84, 1956; (d) Indonesia, *J Trop Pediat*, 2: 199, 1957.
8. METTROP, G. and ROBINSON, P.: Maternal and child health services in S.E. Asia, V. *J Trop Pediat*, 3: 41, 1957.
9. KARUNARATNE, W. A., ROBINSON, P. and TULL, R. L.: Maternal and child health services in S.E. Asia, VI. *J Trop Pediat*, 3: 82, 1957.

Submitted Oct. 26, 1960

Accepted Nov. 10, 1960

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## Identification of Enteropathogenic *Escherichia Coli* 0111:B4 by Means of Fluorescent Antibodies

by DAN DANIELSSON and GUNNAR LAURELL

The bacteriological diagnosis of enteropathogenic *Escherichia coli* by conventional culture and the current serological tests takes 48-72 hours. In other words, the method is time-consuming, and furthermore requires special media. Moody, Goldman & Thomason (11) showed in 1956 that, by means of Coons's (3) fluorescent antibody technique, it is possible to demonstrate pathogenic bacteria within about one hour. This technique has since been used for the identification of a number of different bacteria (1, 4, 5, 12, 13). Whitaker, Page, Stulberg & Zuelzer (18), who examined 4-year-old samples of faeces from an epidemic caused by 0127:B8, demonstrated the superiority of the method for diagnosing enteropathogenic *E. coli*.

In the investigation now described the fluorescent antibody technique was tested during a current epidemic of infantile diarrhoea due to *E. coli* 0111:B4, and was compared with the conventional bacteriological and serological techniques.

### Methods

#### 1. Preparation of Antiserum, and Conjugation with Fluorescent Substance

Rabbit antiserum specific for enteropathogenic *E. coli* 0111:B4 was prepared in

accordance with the directions of Edwards & Ewing (7). The O-agglutinin titre varied from 1:1280 to 1:2560, and the B-agglutinin titre was about 1:124. These sera were used for the serological tests and for conjugation with fluorescent substance. Before conjugation the globulin fraction was precipitated with 33%-saturated ammonium sulphate. The precipitate was dissolved in distilled water, dialysed for 12 hours against running distilled water, and then for a further 24 hours in phosphate-buffered sodium-chloride solution. Conjugation was performed by the method described by Chadwick, McEntegart & Nairn (2), using Lissamine Rhodamine B200 (RB200), which gives a brilliant orange fluorescence. It proved possible to dilute the conjugated globulin solutions 4-5 times with physiological saline without impairing the results. The conjugate was stored in small bottles fitted with screw caps at 4°C, or frozen at -20°C.

#### 2. Treatment of the Smears

Each smear was dried in air, and fixed by heating. A small drop of conjugated globulin solution was spread out so as to cover the smear, and the slide was placed in a damp chamber at room temperature. After a period of 30-45 minutes the slide was washed in phosphate-buffered saline (pH 7.1) for 10-15 minutes. The smear was mounted under a cover-slide in buffered glycerine-saline (pH 7.1). Control tests of positive smears were made on conjugated normal

rabbit globulin, and also by means of inhibition tests (Goldman's one-step procedure (8)).

### 3. Fluorescence Microscopy

A Zeiss fluorescence microscope equipped with dark-field condensor and mercury lamp HBO200 was used. The BG12 ultraviolet input filter in combination with the OG4 or OG5 eyepiece filter (Schott & Gen) gave the best results. The following scale was used for reading off the count of fluorescent bacteria:

Negative	No fluorescent bacteria present
1 +	Isolated fluorescent bacteria present
2 +	About 1 fluorescent bacterium present per field
3 +	2-5 fluorescent bacteria present per field
4 +	5-10 fluorescent bacteria present per field
5 +	Numerous fluorescent bacteria present per field

### 4. Treatment of the Specimens of Faeces

The specimens were as a rule examined 24 hours after collection. Each specimen was treated as described below, and was then stored at  $-15^{\circ}\text{C}$  for subsequent examination (see Fig. 1).

*Modification 1.* Direct culture on Conradi Drigalski's "blue agar plates" (9). After incubation at  $37^{\circ}\text{C}$  for 20-24 hours at least 12-15 colonies were examined by means of the slide agglutination test. Suspect colonies were pure-cultured in broth, and confirmed by means of O-titration in tubes in accordance with current technique.

*Modification 2.* As described by Whitaker *et al.* (14), a small quantity of faeces was suspended in 1 ml of physiological saline in sterile centrifuge tubes. Of this, smears were made on slides measuring about  $2 \times 3$  cm for treatment with fluorescent antibodies and fluorescence microscopy, and direct cultures were also made, as described under Modification 1.

*Modification 3.* A suspension in physiological saline (see Modification 2) was made up to 5 ml, and filtered through gauze to remove coarse particles. The filtrate was centrifuged at 3000 r.p.m. for 10 minutes, and the sediment suspended in about 0.5 ml of physiological saline. Preparations and culture of this were then made, in accordance with the directions under Modification 2 (Thomason *et al.* (13)).

*Modification 4.* A small quantity of faeces was cultured in 2.5 ml of broth at  $37^{\circ}\text{C}$  for 6-8 hours. A direct culture of the broth was made (see Modification 1). The broth was then centrifuged, the sediment suspended in 1 ml of sterile physiological saline, and smears were made for treatment with fluorescent antibodies. Culture of this suspension commonly resulted in such prolific growth that it was difficult to find sufficiently isolated colonies.

The specimens of faeces were again examined after having been stored for 3 months at  $-15^{\circ}\text{C}$ . In these experiments the specimens were first cultured in broth at  $37^{\circ}$  and  $45^{\circ}\text{C}$  for at least 24 hours (see Dixon (6)). The broth culture was then treated as described under Modification 4.

## Material

### 1. The Epidemic in Question

During the autumn of 1959 there was an epidemic of infantile diarrhoea at a Children's Home near Örebro. At the time 26 babies aged from 2 to 12 months were resident at the Home. By the time this investigation was commenced at the end of October 1959 13 of the infants had had diarrhoea for several weeks. *E. coli* strain 0111:B4 was demonstrated among these and in two apparently healthy children, by means of the fluorescent antibody technique. Clinically the epidemic was relatively mild. Three of the children had severe diarrhoea, and were admitted to hospital, where neomycin was given successfully.



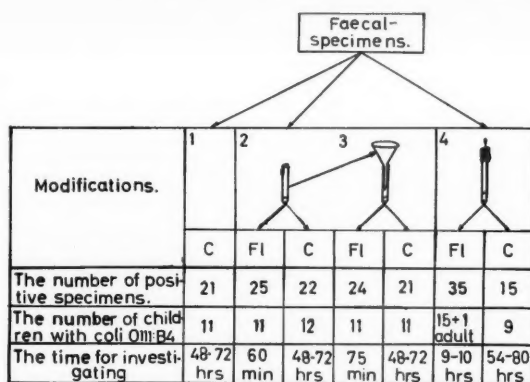


Fig. 1. Occurrence of *E. coli* 0111:B4 according to fluorescent antibody technique and conventional culture with the modifications used in this investigation.

C = Conventional culture. Fl = Fluorescent antibody technique.

## 2. Collection of Specimens

During the first 2 months samples were taken every 4-5 days, and during the last 2 months every 10-12 days. Specimens were obtained from four of the healthy infants on only four occasions, at regular intervals throughout the period of the investigation. The staff, who numbered 24, supplied specimens on two occasions. Altogether 264 specimens of faeces were examined.

## Results

The findings obtained by the various methods of investigation are shown in Fig. 1.

The greatest number of positive results was obtained by the fluorescent antibody technique, as described under Modification 4. Enteropathogenic *E. coli* of strain 0111:B4 were demonstrated in 15 infants on 35 occasions, and in one of the nurses on one occasion. In comparison, the conventional method in the same modification yielded positive results in nine infants on 15 occasions. With regard to other

methods, the fluorescent technique does not differ to any notable extent from conventional procedures. Pathogenic bacteria were demonstrated on slightly more occasions by the fluorescent technique than by conventional methods, however.

The quickest diagnosis was obtained by

TABLE 1. Quantitative distribution of the positive results with fluorescent antibody technique and the number of positive cultures within each group.

C = Conventional culture. Fl = Fluorescent antibody technique.

Modifications (see Fig. 1)	2		3		4	
	Fl	C	Fl	C	Fl	C
Quantitative gradation of bacteria per field	1+ 11	9	12 10	7	0	
	2+ 10	9	10 9	2	1	
	3+ 3	3	1 1	4	2	
	4+ 1	1	1 1	5	0	
	5+ —	—	—	17	12	
No. of positive specimens	25	22	24	21	35	15
No. of children with <i>E. coli</i> 0111:B4	11	12	11	11	15+1 adult	9

TABLE 2. *Quantitative comparison between fluorescent antibody technique, Modification 4 (enrichment), and all the positive cultures together.*

C = Conventional culture. Fl = Fluorescent antibody technique.

Modifications (see Fig. 1!)	Fl 4	C 1, 2, 3, 4
Quantitative gradation of 1 + bacteria per field	7	0
2 +	2	1
3 +	4	4
4 +	5	4
5 +	17	17
No. of positive specimens	35	26
No. of children with <i>E. coli</i> 0111:B4	15 + 1 adult	12

the fluorescent antibody technique without enrichment. It was in this manner possible to establish the presence of *E. coli* 0111:B4 within one hour. With enrichment, which gave the greatest number of positive cultures, this took 9-10 hours. By the conventional procedure it was impossible to obtain a definite diagnosis in less than 48 hours.

Table 1 shows the bacterial counts as obtained by the various modifications of the fluorescent antibody technique, compared with corresponding cultures. It will be apparent that the fluorescent technique is the more sensitive. Several specimens that on culture gave negative results yielded 1+ to 2+ on examination by the fluorescent antibody technique. Only one specimen, which was found by Modification 2 to contain strain 0111:B4 gave negative results with the fluorescent antibody technique. The difference was more striking after culture in broth. In none of the seven cases in which the fluorescent technique gave a result of 1+ were organ-

isms of strain 0111:B4 isolated by culture. Of 17 cases which yielded 5+, 12 gave positive cultures.

Table 2 shows the total number of positive cultures obtained by the various methods, and these are compared with the fluorescent antibody technique, Modification 4, which gave the greatest number of positive results. Altogether, positive results were obtained in nine more cases by the fluorescent technique than by conventional methods. In four of these nine, bacteria of strain 0111:B4 had been obtained by culture on earlier occasions. Enteropathogenic *E. coli* were demonstrated in a further three infants and one adult by the fluorescent antibody technique, compared with the cultures. The number of positive specimens from these cases was thus five.

#### *Antibiotic Therapy*

The importance of antibiotic therapy is apparent from Table 3. All infants at the Home were treated for 5 days. Specimens of faeces were taken from them all on the fourth day of treatment. By standard culture *E. coli* of strain 0111:B4 were isolated from two infants on that occasion. By Modifications 2 and 3, culture procedure, positive results were obtained from three infants, whereas pathogenic bacteria were demonstrated in four by the fluorescent antibody technique. The greatest difference between the methods is seen in Modification 4: conventional culture gave positive results in three cases, whereas the fluorescent procedure revealed pathogenic organisms in nine. After antibiotic therapy no positive cultures were obtained in Modifications 1 and 4, but the fluorescent antibody technique in Modi-

fication 4 revealed the organisms in two infants. In one child *E. coli* of strain 0111:B4 were demonstrated by both techniques in Modifications 2 and 3.

### Semiquantitative Analysis

Laboratory tests were carried out to examine the sensitivity of the fluorescent antibody technique. For this purpose specimens of faeces of a diarrhoea consistency that had yielded positive results in Modifications 1-4 by both techniques were selected. These specimens were diluted with suitable negative faeces, and each dilution was examined by Modifications 1, 2 and 4. The results are collected in Table 4. It can be seen that after culture in broth bacteria were demonstrable by the fluorescent antibody technique in a dilution of 1/128, whereas the greatest dilution after which *E. coli* 0111:B4 could be detected by other techniques was 1/2.

### Survival Power of the Bacteria

All specimens of faeces were re-examined after 3 months' storage at  $-15^{\circ}\text{C}$ . Culture in broth at  $37^{\circ}$  and  $45^{\circ}\text{C}$  was performed before plating out. The results of the final cultures are shown in Fig. 2, from which it is clear that the fluorescent antibody technique yielded a greater number of positive cultures. An unexpected finding is that better results were obtained after incubation of the preliminary cultures at  $37^{\circ}$  than at  $45^{\circ}\text{C}$ .

### Discussion

The fluorescent antibody technique was tested during a current epidemic of

Enrichment in broth for 24 hours	37°		45°	
	FI	C	FI	C
1+	4	2	5	3
Quantitative grad-	5	3	5	4
ation of bac-	3+	3	2	2
teria per field	4+	2	1	1
	5+	17	0	0
The number of positive specimens.	31	27	13	10
The number of children with coli 0111:B4.	15	13	7	6

Fig. 2. Secondary investigation of frozen faecal specimens after primary enrichment in broth at  $37^{\circ}\text{C}$  and  $45^{\circ}\text{C}$  for 24 hours.

C = Conventional culture, FI = Fluorescent antibody technique.

infantile diarrhoea caused by enteropathogenic *E. coli* of strain 0111:B4. After culture in broth it was possible by means of this technique to demonstrate the organisms in 15 infants and one adult, whereas conventional culture revealed them in only 12 infants.

The comparatively small difference between the results of the two methods may possibly be ascribed to the fact that all specimens were taken during an epidemic. Under such circumstances the incidence of bacteria in the faeces is as a rule high, and the organism can readily be demonstrated on culture. When the bacterial count is low the fluorescent antibody technique is probably superior. Evidence in favour of this is supplied by the four positive fluorescence tests in which the strain could not be demonstrated simultaneously, although the organisms had been present in earlier tests; and further support is gained from the results obtained during and after antibiotic therapy. The greater sensitivity of the

TABLE 3. Occurrence of *E. Coli* 0111:B4 according to fluorescent antibody technique and conventional culture during and after treatment with antibiotic.

C = Conventional culture. FI = Fluorescent antibody technique.

Modifications (see Fig. 1)	1		2		3		4	
	C	FI	C	FI	C	FI	C	FI
Positive specimens during treatment	2	4	3	4	3	9	3	
Positive specimens after treatment	0	1	1	1	1	2	0	

fluorescent antibody technique is also confirmed by the laboratory tests performed on various dilutions of samples of faecal material known to contain the strain of bacteria in question.

The fluorescent antibody technique as carried out after enrichment culture gave positive results in nine more cases than did conventional culture, counting all modifications. This may possibly be explained by the fact that the number of bacteria that can be examined on a slide by microscope is greater than the number of colonies one can expect to obtain after inoculation on a plate.

TABLE 4. Laboratorial comparison with dilutions of a known faecal specimen.

C = Conventional culture. FI = Fluorescent antibody technique.

Dilutions	1	2		3	
	C	FI	C	FI	C
1:2	Pos.	2+	Pos.	5+	Pos.
1:4	Neg.	Neg.	Neg.	3+	Neg.
1:8	Neg.	Neg.	Neg.	2+	Neg.
1:16	Neg.	Neg.	Neg.	1+	Neg.
1:32	Neg.	Neg.	Neg.	1+	Neg.
1:64	Neg.	Neg.	Neg.	1+	Neg.
1:128	Neg.	Neg.	Neg.	1+	Neg.
1:256	Neg.	Neg.	Neg.	Neg.	Neg.

With regard to the time taken, the fluorescent antibody technique is clearly superior, and at best a diagnosis can be made within one hour. With conventional culture this takes at least 48 hours. This is undoubtedly of great importance, not least with regard to the quick institution of proper treatment. Furthermore, no media or glassware are required and the diagnosis is thus simplified. The only thing needed is a first-class microscope equipped for fluorescent microscopy, together with good conjugated globulins. The most important disadvantage is that the technique is sensitive, and in order for it to be reliable, requires an experienced operator. At present this would seem to limit the routine use of the method. And it is not known to what extent the diagnosis may be influenced by unspecific reactions to, say common partical antigens to pathogenic coli and other coli bacteria. The results obtained in this investigation do not indicate that this is a serious source of error, however.

Examination of material that had been stored at  $-15^{\circ}\text{C}$  for several months gave results largely similar to those of the primary investigations, and in favour of the fluorescent technique. The results are not absolutely comparable, however, because in the late tests preliminary culture in broth was carried out in all cases. An unexpected feature was the fact that preliminary culture at  $45^{\circ}\text{C}$  produced less good results than that at  $37^{\circ}\text{C}$ . In this respect our findings differ from those of Dixon, who stated that enrichment at higher temperatures yields better results of culture when the enteropathogenic coli count is low.

The direct staining technique was used

in the fluorescent antibody procedure in this investigation. The indirect staining technique is probably more suitable in routine practice, since in the identification of enteropathogenic coli at least 12 different types have to be distinguished; only one conjugated globulin would then be required. As LaBrec, Formal & Schneider (10) have pointed out, however, the indirect method carries a greater risk of unspecific reactions.

### Summary

The fluorescent antibody technique has been tested during a current epidemic of infantile diarrhoea due to *E. coli* 0111:B4,

and has been compared with the conventional bacteriological and serological techniques. Four different modifications have been compared. After enrichment of the faecal specimens in broth the greatest number of positive specimens was obtained by the fluorescent antibody technique. It was possible by means of this technique to demonstrate the organisms in 15 infants and one adult in 35 specimens, whereas conventional culture, all modifications together, revealed them in 12 infants in 26 specimens. The time for putting a diagnosis is greatly reduced by the fluorescent method. The method's disadvantages and its use in routine practice are discussed.

### References

1. CARTER, C. H.: Staining of coagulase-positive Staphylococci with fluorescent antisera. *J Bact*, 77: 670, 1959.
2. CHADWICK, C. S., McENTEGART, M. G. and NAIRN, R. C.: Fluorescent protein tracers. A simple alternative to fluorescein. *Lancet*, 1: 412, 1958.
3. COONS, A. H.: Fluorescent antibody methods. *General Cytochemical Methods*, 1: 399, 1958.
4. DEACON, W. E., PEACOCK, W. L., FREEMANN, E. M. and HARRIS, A.: Identification of *Neisseriae gonorrhoeae* by means of fluorescent antibodies. *Proc Soc Exp Biol Med*, 107: 322, 1959.
5. REPENTIGNY, J. DE and FRAPPIER, A.: Studies of *H. pertussis* liquid cultures. III. Localization of surface antigens by means of fluorescent antibody. *Canad J Microbiol*, 2: 677, 1956.
6. DIXON, J. M. S.: Observations on the growth in nutrient broth of mixtures of *Escherichia coli* strains and of faecal specimens harbouring pathogenic *Escherichia coli* serotypes. *J Hyg*, 57: 174, 1959.
7. EDWARDS, P. R. and EWING, W. H.: Identification of Enterobacteriaceae. Burgers Publishing Companies, 1957.
8. GOLDMAN, M.: A new serologic test for toxoplasma antibody using fluorescein-tagged globulin. *Amer J Trop Med*, 5: 375, 1956.
9. KAUFFMANN, F.: Enterobacteriaceae, 2nd ed., 1954. Munksgaard, Copenhagen.
10. LABREC, E. H., FORMAL, S. B. and SCHNEIDER, H.: Serological identification of *Shigella flexneri* by means of fluorescent antibody. *J Bact*, 78: 384, 1959.
11. MOODY, M. D., GOLDMAN, M. and THOMASON, B. M.: Staining bacterial smears with fluorescent antibodies. I. General methods for *M. pseudomallei*. *J Bact*, 72: 357, 1956.
12. MOODY, M. D., ELLIS, E. C. and UPDYKE, E. L.: Staining bacterial smears with fluorescent antibody. IV. Grouping Streptococci with fluorescent antibody. *J Bact*, 75: 553, 1958.
13. THOMASON, B., CHARRY, W. B. and EDWARDS, Ph. R.: Staining bacterial smears with fluorescent antibody. VI. Identification of Salmonellae in faecal specimens. *J Bact*, 77: 478, 1959.
14. WHITAKER, J., PAGE, R. H., STUHLBERG, C. S. and ZUELZER, W.: Rapid identification of enteropathogenic *Escherichia coli* 0127:B8 by the fluorescent antibody technique. *Amer J Dis Child*, 95: 1, 1958.

Submitted Dec. 5, 1960

Accepted Dec. 8, 1960

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## Serial Blood-Gas Tensions and Acid-Base Balance during the First Hour of Life in Human Infants<sup>1</sup>

by T. K. OLIVER, JR., J. A. DEMIS<sup>2</sup> and G. D. BATES

Although there have been a number of biochemical studies performed on umbilical cord blood samples, very few serial studies have been made during the first hour of life—the period in which many of the critical respiratory and circulatory adaptations to extrauterine life occur (22).

In the present investigation acid-base balance of arterial blood has been followed serially with studies beginning within five minutes of delivery in the majority of subjects and continuing through the first hour. In addition, the partial pressures of oxygen ( $\text{PaO}_2$ ) and carbon dioxide ( $\text{PaCO}_2$ ) have been directly determined. It was hoped that  $\text{PaO}_2$  in particular would add to the knowledge of this important period since, in general, it more precisely defines hypoxemia than does oxyhemoglobin saturation and thus might give information regarding the effectiveness of the lungs to oxygenate the blood as well as the presence of extra pulmonary right to left shunts.

### Material and methods

Forty infants on the clinical obstetrical

<sup>1</sup> Supported in part by a grant from the Comley-Coleman Fund of the Ohio State University.

<sup>2</sup> Medical student-research fellow (Jan-April 1960) of The Central Ohio Heart Association.

service of the Ohio State University Health Center were studied. All except three were mature by birth weight (these 3 weighed 2495, 2407 and 2280 g). In 27 the pregnancy, labor and (vertex) delivery were considered entirely normal: 11 mothers had forceps applied at the outlet to assist delivery, while in the remainder (16) delivery was spontaneous. Nine infants were delivered by elective cesarean section and four mothers had abnormalities either in maternal health (2) or in presentation (2). With few exceptions the mothers received a combination of meperidine and phenergan i.m. or i.v. during labor. The usual dose was 50 mg of each and, with few exceptions, they received this only once. No differences in results were observed in the groups who did or did not receive analgetic drugs. Anesthesia for the vertex deliveries consisted of inhalation ( $\text{N}_2\text{O}$  or GOE) in 15, conduction (spinal or caudal) in 10, local or none, 1 each. All the patients delivered by cesarean section received conduction anesthesia. All infants were rated by the Apgar scoring method one minute after complete delivery (1).

Immediately after delivery the umbilical cord was doubly clamped and ligated. The cord was then successively swabbed with an organic iodide preparation (Virak) and alcohol and then was amputated within 2 cm of the abdominal wall, in the meanwhile having an assistant compress the cord at the abdominal wall to prevent bleeding. After shallow dilatation of one of the arteries a



catheter<sup>1</sup> was inserted a distance of 4–10 cm. When it was technically not possible to cannulate an artery an attempt was made to catheterize the left atrium through the umbilical vein using a soft, round-tip polyethylene catheter marked at 1 cm intervals from 10–15 cm. Since this was a blind procedure, although recognized as technically easy because of the proximity of the foramen ovale to the inferior vena cava (10), it was possible to be certain that left atrial blood was obtained only when the catheter was 12–15 cm from the abdominal wall and the sample had a distinctly brighter color than blood obtained after withdrawing the catheter 1–2 cm. The results of left atrial samples are therefore somewhat weighted. Rectal temperature was recorded immediately after each sample was obtained.<sup>2</sup>

The protocol called for a sample in the first five minutes of life (successful in 24 infants) and serially thereafter at 10, 20, and 60 minutes. In fact, there was considerable deviation from the protocol and the results were grouped into the time periods 2–5, 6–10, 11–20, 21–40, and 41–64 minutes. Twenty-eight infants had at least 3 samples withdrawn, 9 had 2 and 3 infants only 1.

Each sample, 1.5–2.0 ml in volume, was collected in a lightly oiled syringe, the dead space of which was filled with heparin. A small amount of mercury was added after collection to assure mixing, following which the syringe was placed in crushed ice until analysis. Previous studies in this laboratory have shown that gas tensions, pH and CO<sub>2</sub> content are unaffected by adding heparin or mercury. The blood gas tensions, pH, CO<sub>2</sub> content and hematocrit determinations were begun within  $\frac{1}{2}$ –2 hours after collection. The sera were frozen under mineral oil for subsequent determination of the concentration of chloride, sodium, potassium, magnesium and total protein. All chemical determinations were performed in duplicate. Blood

<sup>1</sup> A No. 5 ureteral catheter was found to be most satisfactory. Polyethylene catheters tend to kink during insertion because of arterial spasm.

<sup>2</sup> The senior author is indebted to Dr. L. S. James who discussed and demonstrated the umbilical catheterization techniques with him.

gas tensions were determined by a micro-modification of the blood-bubble equilibration technique (4). Carbon dioxide content and pH were determined on plasma, obtained by centrifuging blood under a thick layer of neutral mineral oil. A Kopp-Natelson microgasometer was used for determining CO<sub>2</sub> content (25). Plasma pH was determined at 37.5°C. using a modification of the Beckman microglass capillary and a Beckman model GS pH meter (47). Sodium and potassium were analyzed on a flame photometer with an internal lithium standard; chloride was determined by a titrimetric method (34); magnesium by a micromodification of the method of Basinki (3); total protein by the biuret method (14).<sup>3</sup> Electrolyte determinations were not performed on hemolyzed specimens. Sera of known chemical composition<sup>4</sup> were analyzed with each batch of patient's sera.

### Calculations

The partial pressures of O<sub>2</sub> and CO<sub>2</sub> were calculated to dry, ambient pressure, T 37.5°C then corrected to body temperature (7). Oxygen saturation was obtained from the fetal oxyhemoglobin dissociation curve (31) using the corrected PO<sub>2</sub>. The pH values were corrected to body temperature using Rosenthal's factor for whole blood (0.0147 units/°C). Calculated PCO<sub>2</sub>, to compare with direct PCO<sub>2</sub>, was derived from the Henderson-Hasselbalch equation; pK' was determined from the nomogram of Severinghaus *et al* (35). Buffer base (38) or "base excess" (2), which are useful methods of assessing metabolic disturbances, were not calculated in these infants for two reasons. Hematocrits, in general, were performed only on the initial and final blood samples and then not consistently. Secondly, the Singer-Hastings nomogram for calculating buffer base assumes a serum protein concentration of 7.2 g/100 ml, a pK' 6.11 for carbonic acid and a

<sup>3</sup> Details of all methods (except Mg) are available in the Manual of Clinical Chemistry, the Children's Hospital, Columbus, Ohio, S. Meites, Ph.D., editor.

<sup>4</sup> Lab-trol, Dade Reagents Inc., Miami, Fla.



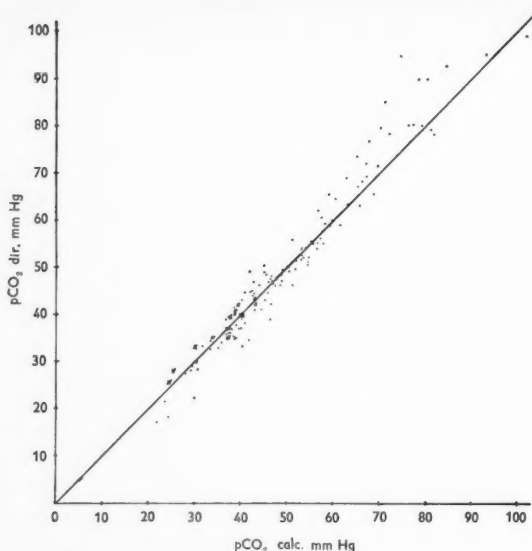


Fig. 1. Comparison of direct with calculated  $\text{PaCO}_2$ . Solid line is the line of identity. Dots (·) infants in present study. Crosses (×) infants, children and adults (4). A close correlation below  $\text{PaCO}_2$  65 mm Hg is present.

$\text{CO}_2$  solubility factor of 0.0311. All three assumptions tend to be incorrect during the neonatal period: serum protein concentration is reduced; marked variations of  $\text{pK}'$  and solubility occur as a result of temperature and pH shifts, which of course, may be profound during the first minutes and hours of life. It is believed that the tabular plus the graphic (9) representation of the relationship of pH,  $\text{PCO}_2$  and  $\text{CO}_2$  content are as helpful in these circumstances as buffer base. Attention is also drawn to the fact that slight loss of  $\text{CO}_2$  from the plasma through the overlying mineral oil may occur at high  $\text{CO}_2$  tensions; this will result in a decrease of calculated  $\text{PCO}_2$  compared to direct  $\text{PCO}_2$ . This does not occur when the  $\text{PCO}_2$  is less than 65 mm Hg (Fig. 1).

### Results (Table 1)

#### *Blood Gas Tensions*

As expected, the partial pressure of oxygen was very low and carbon dioxide

markedly elevated at birth. During the succeeding minutes the values tended to approach normal limits, although wide variations occurred in both  $\text{PaO}_2$  and  $\text{PaCO}_2$  during the entire hour (Figs. 2 & 3). No statistical difference was found in the  $\text{PaO}_2$  of infants born by vertex versus those delivered by cesarean section except at the 11–20 minute period (Table 3).

The  $\text{PaO}_2$  in left atrial blood was uniformly higher than from the artery. In part this represents the biased selection of LA samples but it also clearly indicates the presence of a right to left shunt through the ductus arteriosus. This was documented in one instance when samples were simultaneously obtained from LA and artery with values of 72.6 and 57.4 mm Hg, respectively.

Only four oxygen tensions were above

TABLE 1. *Serial biochemical data during the first hour of life.*

Determination		Time period (minutes)				
		2-5	6-10	11-20	21-40	41-64
PaO <sub>2</sub> mm Hg	No.	23	22	28	12*	29
	Mean	19.5	48.7	56.3	56.7	61.7
	SD	12.3	15.9	12.1	7.7	13.8
	Range	0.6-47.3	29.7-88.1	37.5-79.8	49.2-72.5	40.1-89.5
PaCO <sub>2</sub> mm Hg	No.	24	22	31	24	30
	Mean	76.4	57.2	46.3	39.2	38.0
	SD	11.6	13.0	8.3	7.4	6.9
	Range	55.4-98.8	40.0-94.9	28.5-79.0	21.7-55.0	18.4-56.3
pH	No.	23	21	31	24	30
	Mean	7.10	7.19	7.25	7.31	7.34
	SD	.09	.08	.10	.08	.06
	Range	6.94-7.22	7.00-7.30	6.94-7.43	7.14-7.52	7.23-7.45
CCO <sub>2</sub> mM/L	No.	24	21	31	24	30
	Mean	24.5	23.3	20.8	20.6	21.6
	SD	2.8	2.9	2.6	2.4	2.3
	Range	17.4-29.3	15.6-26.6	14.7-24.8	16.1-23.7	14.6-26.7
Na mEq/L	No.	19	18	21	18	24
	M	139	136	135	135	134
	SD	8	7	5	4	5
	R	130-158	122-148	120-141	130-145	120-140
K mEq/L	No.	19	18	21	18	24
	M	9.1	8.4	8.0	8.5	8.0
	SD	2.0	1.8	1.8	1.7	1.8
	R	6.8-14.3	5.9-12.0	5.6-12.7	5.4-11.4	5.3-11.4
Cl mEq/L	No.	11	10	13	13	20
	M	107	107	109	108	109
	SD	4	4	5	3	4
	R	99-110	102-111	98-115	102-113	101-113
Mg mEq/L	No.	5	2	6	9	14
	M	2.0	2.1	2.5	1.8	2.0
	SD	—	—	—	0.5	0.5
	R	1.4-2.7	2.0-2.2	2.0-2.9	1.6-2.9	0.9-2.5
Total Protein gm/100 ml	No.	18	15	18	16	22
	M	6.4	6.2	6.4	5.9	6.1
	SD	0.6	0.6	0.8	0.6	0.9
	R	5.3-7.3	5.2-7.3	5.7-8.7	5.0-7.7	4.8-7.0
Hematocrit %	No.	14	—	—	—	15
	M	51	—	—	—	50
	SD	5	—	—	—	4
	R	38-58	—	—	—	41-57

\* 9 LA samples not included.

the lower limit of normal for this laboratory (88 mm Hg). Two of these were LA samples and two were from the artery. Ten values were greater than 75 mm Hg and these were restricted to six infants

thus demonstrating the variability with which this phase of extrauterine adaptation occurs.

During the period 21-40 minutes nine of the twenty-one samples were from the left

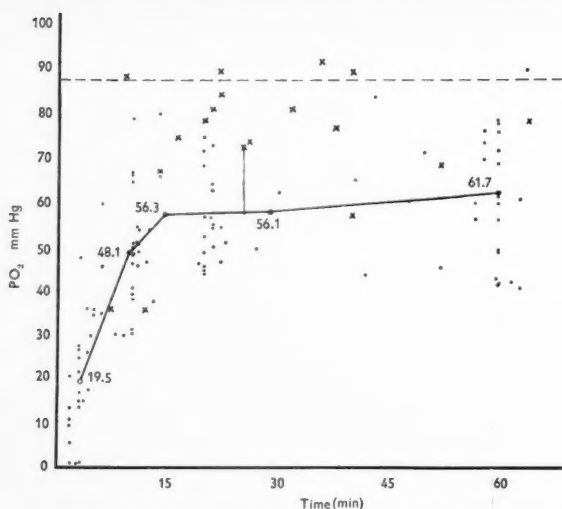


Fig. 2.  $\text{PaO}_2$  versus time. The figures are the means of the five time periods. The dots indicate arterial samples. The crosses indicate left atrial samples. The dashed line is the lower limit of normal  $\text{PaO}_2$  for older children and adults (88 mm Hg).

TABLE 2. Acid-base balance in infants 1-3 days of age. (Mean value.)

Author	pH	$\text{CO}_2$ mM/L	$\text{pCO}_2$ mm Hg
Graham & Wilson	7.43	19.7	30.0
Cook <i>et al.</i>	7.44	21.9	31.2
Stahlman	7.43	21.9	32.7
Weisbrot <i>et al.</i>	7.41	21.4	33.6
Reardon <i>et al.</i>	7.39	21.1	34.1

atrium (Fig. 2). For this reason these values were not included in the calculation of the mean  $\text{PaO}_2$  for this time period (Table 1). During the other periods the atrial values were included since they were few in number and did not significantly alter the mean. The mean  $\text{PaO}_2$  at 2-5 minutes was  $19.5 \text{ mm Hg} \pm 12.3$ . Although the lowest observed value (0.6 mm Hg) occurred in an infant whose mother required heavy sedation, values of 0.8 and 0.9 mm Hg were observed fol-

lowing entirely normal vertex deliveries. The two highest values, 47.3 and 35.5 mm Hg, occurred following vertex and section deliveries, respectively.

During the 6-10 minute period the largest increment of change in mean values occurred.  $\text{PaO}_2$  rose to a mean of 48.7 mm Hg, an increase of 29.2 mm Hg. During the same period  $\text{PaCO}_2$  fell 19.2 mm Hg, to a mean of 57.2 mm Hg. During the next ten minutes  $\text{PaO}_2$  rose to a mean of 56.3 mm Hg and  $\text{PaCO}_2$  fell to a mean of

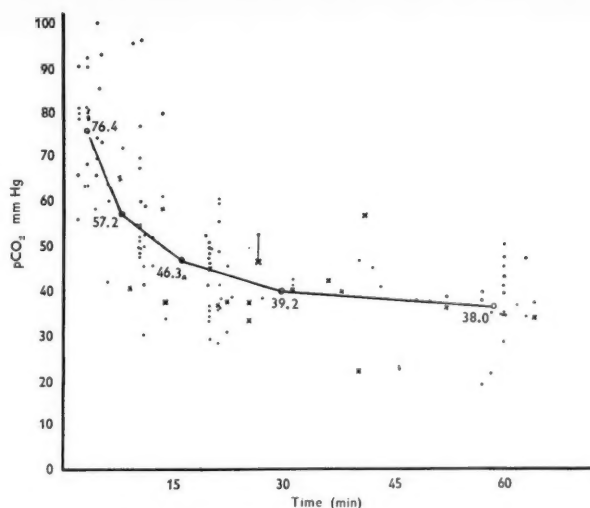
Fig. 3.  $\text{PaCO}_2$  versus time. Symbols as in Figure 2.

TABLE 3. Serial acid-base balance in infants delivered by vertex and by cesarean section.

	2-5		6-10		11-20		21-40		41-64	
	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (SD)
$\text{pCO}_2$ mm Hg										
Vaginal	16	74.4 ( $\pm 9.4$ )	17	54.0 ( $\pm 9.9$ )	20	41.9 ( $\pm 9.2$ )	16	40.6 ( $\pm 7.2$ )	21	37.1 ( $\pm 6.9$ )
C.S.	6	81.3 ( $\pm 15.7$ )	3	58.9 ( $\pm 10.9$ )	8	56.6 ( $\pm 9.9$ )	6	37.0 ( $\pm 8.6$ )	7	41.2 ( $\pm 7.8$ )
P		$0.1 > p > 0.05$		$> 0.1$		$< 0.01$		$> 0.1$		$> 0.1$
pH										
Vaginal	15	7.13 ( $\pm 0.07$ )	16	7.21 ( $\pm 0.07$ )	20	7.29 ( $\pm 0.07$ )	16	7.31 ( $\pm 0.06$ )	21	7.35 ( $\pm 0.05$ )
C.S.	6	7.04 ( $\pm 0.05$ )	3	7.13 ( $\pm 0.08$ )	8	7.15 ( $\pm 0.12$ )	6	7.34 ( $\pm 0.12$ )	7	7.31 ( $\pm 0.08$ )
P		$< 0.05$		$0.1 > p > 0.05$		$< 0.01$		$> 0.1$		$> 0.1$
$\text{CCO}_2$ mM/L										
Vaginal	16	25.1 ( $\pm 2.3$ )	17	22.2 ( $\pm 2.2$ )	20	20.9 ( $\pm 2.5$ )	16	21.1 ( $\pm 2.3$ )	21	21.5 ( $\pm 2.6$ )
C.S.	6	22.3 ( $\pm 3.1$ )	3	20.6 ( $\pm 4.6$ )	8	20.4 ( $\pm 3.4$ )	6	20.6 ( $\pm 2.1$ )	7	21.7 ( $\pm 2.1$ )
P		$< 0.05$		$> 0.1$		$> 0.1$		$> 0.1$		$> 0.1$
$\text{pO}_2$ mm Hg										
Vaginal	15	22.0 ( $\pm 12.8$ )	17	52.2 ( $\pm 16.1$ )	19	59.0 ( $\pm 12.3$ )	15	66.6 ( $\pm 6.4$ )	20	62.8 ( $\pm 19.8$ )
C.S.	6	16.0 ( $\pm 10.3$ )	3	38.0 ( $\pm 13.6$ )	7	47.1 ( $\pm 8.3$ )	6	55.7 ( $\pm 4.0$ )	7	57.7 ( $\pm 13.1$ )
P		$> 0.1$		$> 0.05$		$< 0.05$		$> 0.05$		$> 0.1$

46.3 mm Hg. During the remainder of the first hour there were only slight changes in these values; mean  $\text{PaO}_2$  rose to 61.7 mm Hg and  $\text{PaCO}_2$  fell to 38.0 mm Hg at one

hour of age. The calculated mean percentage oxyhemoglobin saturations during these periods were 27, 83, 90, 92, and 95 %.

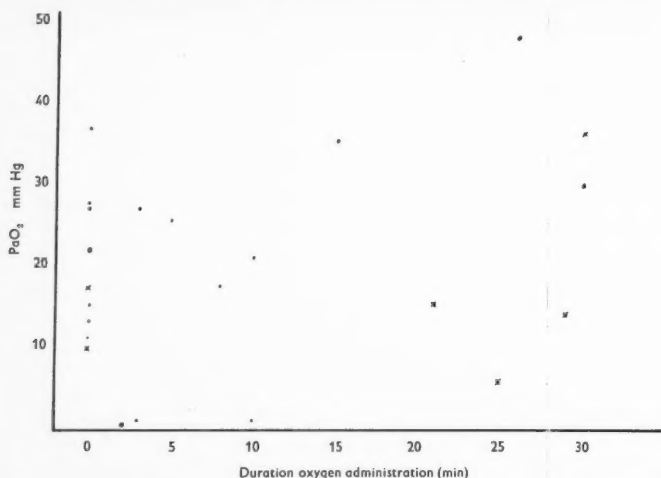


Fig. 4. The relationship of continuous administration of oxygen to mothers upon their infant's  $\text{PaO}_2$  within 5 minutes of birth. No effect is apparent. Symbols: (·), normal vertex delivery; (×) cesarean section; (○) abnormality in pregnancy or delivery.

#### *The effect of administration of oxygen to mothers*

Blood was obtained within 5 minutes of delivery in fourteen infants whose healthy mothers received oxygen by face mask continuously for periods of 2 to 30 minutes prior to delivery. Their  $\text{PaO}_2$  was compared with nine infants whose mothers received no  $\text{O}_2$  (Fig. 4). No effect of maternal hyperoxygenation upon the infant's oxygen tension was found.

#### *Acid-base balance*

In considering acid-base balance it is important to remember that both neonates (8, 15, 30, 41, 45) and pregnant women (6, 26, 44) have a reduction in  $\text{PaCO}_2$ ,  $\text{CO}_2$  content and buffer base. Thus, by normal adult standards these individuals would be considered to have a mixed metabolic acidosis and respiratory alkalosis. The present results must therefore be compared with normal neonates values, not those of

adults. Values of pH,  $\text{CO}_2$  content and  $\text{PCO}_2$  for healthy infants one to three days of age obtained by several investigators are shown in Table 2.

Differences in acid-base balance between infants delivered vaginally and by cesarean section were noted during the first 20 minutes (Table 3, Fig. 5). The mean values of the twenty-nine normal infants indicated an essentially pure respiratory acidosis which continued to improve during the hour, although improvement was principally achieved in the first 20 minutes. Even at one hour, however, the  $\text{PaCO}_2$  was still above normal. In contrast to these infants, the infants born by cesarean section had a moderate metabolic acidosis in addition to a somewhat more severe respiratory acidosis. Furthermore, the pattern of recovery was delayed and it was not until 21–40 minutes that the mean values approached the vertex-delivered infants.

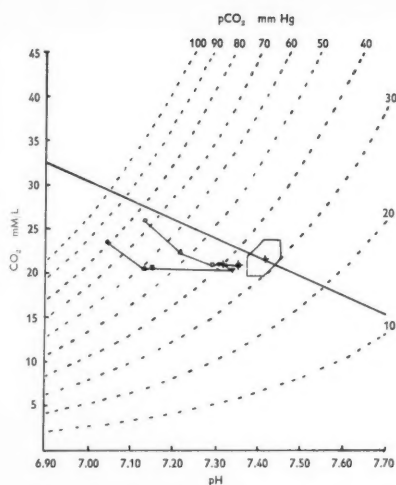


Fig. 5.

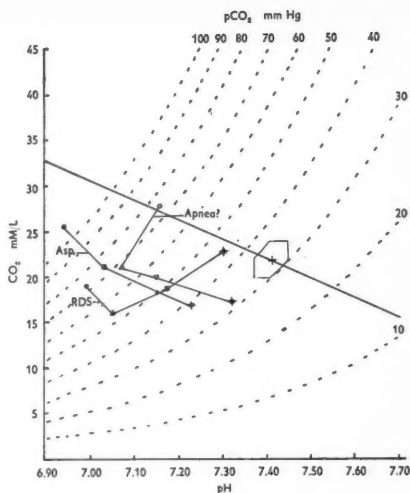


Fig. 6.

Fig. 5. Serial acid-base balance in normal vertex (open symbols) and cesarean section deliveries (solid symbols). The cross (+) is the normal mean for infants 1-3 days of age and the surrounding hexagon represents 1 standard deviation. The symbols indicate time periods: (O), 2-5; (Δ), 6-10; (□), 11-20; (▽), 21-40; and (⊕), 41-64 minutes.

Fig. 6. Serial acid-base balance in 3 infants with post partum respiratory distress. Abbreviations: RDS, respiratory distress syndrome; Asp, aspiration of amniotic fluid; Apnea?, apnea of unknown origin. Symbols as in Figure 5.

#### *Electrolytes, protein, and hematocrit*

No significant differences in the concentration of serum electrolytes were noted between infants born by vertex or cesarean section. No changes with time were found in the concentrations of chloride, sodium, or magnesium. Serum chloride values were higher than adults, an observation previously noted by Graham *et al* (16). Serum potassium levels were strikingly elevated by adult standards, the mean at 2-5 minutes being 9.1 mEq/L, falling at 41-64 minutes to 8.0 mEq/L. The range of values was wide and a few extremely high ones were noted. For example, during the first five minutes 6 infants delivered by vertex had a concentration greater than 10.0 mEq/L; two of these had reduced

Apgar scores (5 & 6) but the remaining four infants had scores of 8 or higher. The serum magnesium values were consistently within the range of adult normal (1.5-3.0 mEq/L).

The mean serum total protein concentration for the entire group of children ranged from 6.4 g/100 ml at birth to 6.10 g/100 ml at one hour. The infants born by vaginal delivery had a significantly higher protein concentration during all except the 21-40 minute period than infants born by cesarean section (Table 4).

The mean value for hematocrit during the initial and final periods were 51 and 50 %, respectively. Unfortunately too few determinations were performed in the cesarean group for statistical comparison

TABLE 4. Serum protein concentration in vertex and cesarean section deliveries (g/100 ml).

Time period (min)	Vertex	C.S.	P
2-5	6.7 ( $\pm 0.42$ )	5.6 ( $\pm 0.36$ )	< 0.01
6-10	6.5 ( $\pm 0.45$ )	5.3 ( $\pm 0.16$ )	< 0.01
11-20	6.7 ( $\pm 0.73$ )	5.6 ( $\pm 0.35$ )	< 0.05
21-40	6.1 ( $\pm 0.60$ )	5.5 ( $\pm 0.54$ )	0.1 > $p$ > 0.05
41-64	6.4 ( $\pm 0.38$ )	5.1 ( $\pm 0.38$ )	< 0.01

TABLE 5. Comparison of biochemistries in infants with Apgar scores of 5-7 and 8-10. (Mean values.)

		Time period (minutes)			
	Score	2-5	6-10	11-20	41-64
PaO <sub>2</sub>	5-7	15.1	45.0	51.5	63.9
mm Hg	8-10	22.8	51.9	57.5	60.8
PaCO <sub>2</sub>	5-7	78.8	63.0	45.5	35.3
mm Hg	8-10	74.7	51.7	46.4	39.0
pH	5-7	7.08	7.15	7.23	7.35
	8-10	7.11	7.22	7.25	7.33
K	5-7	9.8	8.7	7.9	8.3
mEq/L	8-10	8.9	7.9	8.0	7.9

since a difference from the normal infants was suggested. In the former group the values of the three hematocrits performed during the first 5 minutes were 38, 46, and 49 %; in 11 infants born by vertex presentation all except two (with values of 48 and 50 %) had values higher than 51 % at the 2-5 minute period.

#### *Relationship of biochemistries to Apgar score*

Thirty-nine of the 40 infants had scores of 5 or higher. Twenty-eight had scores of 8-10; these included 20 vertex deliveries, 5 infants of cesarean section and 3 with history of abnormalities during pregnancy or delivery. Eleven infants had scores of 5-7; seven were vertex and 4

were section deliveries. Differences in mean values of PaO<sub>2</sub>, acid-base balance and serum potassium concentration were noted in these two groups during the first 10 minutes of life (Table 5). By 11-20 minutes and thereafter no differences were apparent.

#### *Discussion*

Knowledge of the arterial oxygen tension is important primarily because the level indicates the head of pressure which forces oxygen into the tissues. The value cannot be derived accurately from the O<sub>2</sub> saturation at low or, particularly, high saturations (90 % or higher) because of the shape of the oxyhemoglobin dissociation curve.



tion curve. Furthermore, unless body temperature and pH are taken into account large errors may be made by such calculations (36). This is very important in the period immediately following birth when both temperature and pH may be greatly altered.

The importance of knowing the oxygen tension is well illustrated by the values observed at 1 hour of age. Despite the fact that the mean oxyhemoglobin saturation was within normal limits (95 %) the infants nonetheless had moderate hypoxemia as indicated by the mean  $\text{PaO}_2$  (61.7 mm Hg).

Comparatively few investigations of oxygen tension have been performed in neonates and no serial studies have been reported. Sjöstedt *et al* (39) and Beer *et al* (5) determined the  $\text{PaO}_2$  from the clamped umbilical cord following normal vaginal deliveries, and found mean values of 16.9 and 9.2 mm Hg, respectively. No studies are available to compare with the levels obtained during the first hour. Graham (17) has determined  $\text{PaO}_2$  in infants 3 to 54 hours of age. He found a mean of 74 mm Hg (versus his normal for adults of 84 mm Hg). Reardon *et al* have recently reported (30) the results of arterial oxygen tensions on 24 infants ranging in age from 3 to 135 hours. They found a mean value of 82.7 mm Hg. The mean value for adults by the method these investigators employed is approximately 95 mm Hg. Thus it is apparent that there is a significant reduction in oxygen tension for several days of life even though the percentage saturation of oxyhemoglobin approaches or becomes normal promptly, in some cases as early as 10 minutes after birth. There appear to be two reasons for

the low tension; shunting through the ductus arteriosus and shunting within the lungs.

Although the principle direction of the shunt through the ductus arteriosus, which is physiologically patent for several days (42), is from left to right, there is also some R-L shunt. This has been demonstrated angiocardigraphically (18) as well as chemically. Eldridge & Hultgren (12) found a difference in oxygen saturation in arterialized capillary finger and heel blood during the first three days of life, although these infants were deliberately stimulated to cry, thus promoting a R-L shunt. Our studies show that left atrial samples have a consistently higher oxygen tension than arterial samples. This is true whether the infants are quiet or crying. The only conclusion permitted by these results is a right to left shunt through the ductus. However this does not explain the finding that there were only four values within the range of adult normal. The most likely explanation for this is a disturbance of pulmonary ventilation-perfusion relationships ("pulmonary shunting"), presumably due to uneven ventilation. Graham has supporting evidence for this belief (17). Neonates and adults were given 50 % oxygen to breathe: the mean  $\text{PaO}_2$  of the adults rose to 270 mm Hg, whereas the infant's rose to only 153 mm Hg. Furthermore, since Reardon *et al* collected temporal artery samples a shunt through the ductus arteriosus cannot explain their reduced values.

The decreased  $\text{PaO}_2$  is not due to disturbance in diffusion since this has been shown to be normal in newborns (41). A right to left shunt through the foramen ovale appears to be an unlikely explanation

for the reduced  $\text{PaO}_2$ . Although there is evidence (18, 23, 28) that a shunt occurs in some neonates during crying it has not been observed during quiet periods. We have made observations of  $\text{PaO}_2$  during quiet followed by forced cry in 9 infants. In three there was a significant rise of  $\text{PaO}_2$  (6, 14, 15 mm Hg) with crying; in three there was no change and in three a decrease of 10–18 mm Hg. These results suggest that changes occurring with cry are due to changes in ventilation as well as possible shunting through the foramen ovale or the ductus arteriosus.

Many obstetricians and anesthesiologists practice and teach the principle of having mothers breathe high oxygen concentrations during the second stage of labor with the view to increasing the oxygenation of the fetus and the newborn. Sometimes cited as evidence is the study of McClure (24) who observed a higher oxygen tension (mean 10 mm Hg) in umbilical venous blood in a group of infants whose mothers received oxygen compared to a control group. The objection to this study is that umbilical venous rather than arterial blood was analyzed. It is, of course, the latter that perfuses the tissues. Our study of oxygen tensions and James' of oxygen saturation (19) in arterial blood clearly demonstrate that continuous maternal oxygen administration of up to one hour's duration does not increase the infant's  $\text{PaO}_2$  at birth. Whether or not the fetus is better oxygenated by  $\text{O}_2$  therapy cannot be answered by these studies.

The failure of maternal hyperoxygenation to effect the infant particularly when it is considered with the acid-base disturbance at birth appears to be best explained by the concept that the delivery process

results in asphyxiation (20, 21). In the normal infants delivered by vertex presentation the acid-base changes are essentially those of a pure respiratory acidosis. In depressed infants and, in our material, infants born by cesarean section, a metabolic acidosis is superimposed. In the normal babies the recovery from the acidosis was very prompt—occurring primarily in the first 10 minutes of life. In the section babies it took 21–40 minutes. James has pointed out that the recovery process is slower in more severely depressed infants. It may not only be slower but may assume a different pattern as well. Three infants who had serial blood collections developed respiratory disturbances. One of these developed, beginning at 20 minutes of age, typical clinical and, later roentgenological evidence of the respiratory distress (hyaline membrane) syndrome. At birth his acid-base balance was characterized by the most profound metabolic acidosis we observed in any of the infants in the study (Fig. 6). His initial ventilatory response, as evidenced by a rise in  $\text{PaO}_2$  and fall in  $\text{PaCO}_2$  was quite normal and it was only at 1 hour that chemical evidence of disturbed ventilation was noted, by which time he had nearly recovered from the metabolic acidosis. This infant had mild clinical manifestations and was completely normal at 24 hours of age. In contrast to this infant, 2 others with respiratory depression at birth (one due to aspiration of amniotic fluid, the other of unknown cause) had a pure respiratory acidosis at birth; metabolic acidosis occurred some minutes later (Fig. 6), presumably as a result of a somewhat prolonged period of respiratory depression even though both infants were resuscitated

beginning within 2 minutes of delivery. Although the data are suggestive, more studies beginning at birth in infants who subsequently develop evidence of the respiratory distress syndrome are necessary before one can conclude that the primary disturbance is metabolic rather than respiratory.

The finding of hyperkalemia during the first hour of life adds to rather than settles the confusion on this point. James (21) and Reardon (29) have found only a slight elevation of potassium at birth in normal infants which is said to return to within normal limits within one hour although details have not been reported. Earl *et al* (11) reported a mean K concentration of 5.0 mEq/L in fresh placental venous blood. In contrast, Widdowson & McCance (45) observed a mean value of 8.0 mEq/L (range 4.8–12.9) in umbilical venous blood obtained immediately after delivery. These discrepancies suggest technical errors and, indeed, James has observed that the potassium concentration of umbilical venous blood obtained from the placenta rises sharply if there is a delay before the sample is obtained. However, this objection does not apply to these studies. It is well established that a fall in pH results in a shift of potassium from the cells to the extracellular space and a consequent rise in serum concentration (13, 32, 37). This is true whether the reduction in pH is metabolic or respiratory in origin. When pH is controlled profound changes in  $\text{PCO}_2$  or  $\text{CO}_2$  content do not cause a potassium shift (37). It is therefore not remarkable that serum potassium concentration should be elevated in newborns. However it has been shown in animals that it takes 1–2 hours for

peak serum levels to be reached after the initiation of acute acidosis and this observation does not fit with the hypothesis that the period of asphyxiation is of short duration. Perhaps it is longer than we presently believe.

Within the first hour potassium concentration begins to fall as the pH rises. This effect has been shown to occur in dogs in whom the ureters have been ligated and is due to a shift of potassium back into the cells (32). Pincus *et al* have observed modest elevation in potassium concentration (means of 6.1–5.4 mEq/L) in premature infants of varying weight groups in the first 24 hours of life (27). Furthermore, in infants 1–9 days of age the mean level was 4.9 mEq/L and in only 2 to 15 infants was the concentration below the adult mean of 4.4 mEq/L (11). It thus appears that it may take several days for potassium to reach adult levels from the high levels noted at birth and in the first hour of life. Insofar as none of the infants manifested clinical evidence of hyperkalemia it is possible that they were protected by their coexisting hypercarbia (33).

Usher (43) has emphasized that hyperkalemia is a feature of the neonatal respiratory distress syndrome and has suggested a plan of management (44). His finding fits in well with the observations of a metabolic acidosis with further lowering of pH in this syndrome.

Several differences between infants born vaginally and by cesarean section have been observed in this study. The decreased protein concentration in infants born by section has also been found by Reardon (29). The mechanism of this finding is unknown although she has suggested it

is due to hemodilution. A lower hematocrit in these infants is not entirely unexpected since factors which appear to be operative in the "placental transfusion" including uterine contractions (39) and elastic recoil of the chest (22) are not operative in section infants. In addition, if hemodilution does in fact occur in these infants it would contribute to the lower values. Further study is clearly indicated.

### Summary

1. Serial determinations of oxygen and carbon dioxide tension, pH,  $\text{CO}_2$  content, sodium, potassium, chloride, magnesium, total protein and hematocrit beginning within 5 minutes of birth and continuing through the first hour have been performed in newborn infants.

2. The data support the concept that the delivery process is invariably asphyxiating whether delivery is vaginal or by cesarian section. Furthermore, the major recovery occurs within 10 minutes of delivery in healthy infants.

3. Arterial oxygen tension was reduced throughout the entire period. Even though the mean arterial oxyhemoglobin saturation

was within normal limits at one hour of age the mean oxygen tension remained low. The interpretation of these findings is discussed.

4. The administration of high oxygen concentration to the mothers for periods up to 30 minutes failed to effect the infants'  $\text{PaO}_2$ .

5. Evidence is presented for a right to left shunt through the ductus arteriosus when the newborn is quiet as well as crying.

6. Serum potassium concentration was found to be considerably elevated in all infants. The mechanisms for this are discussed.

7. Differences in acid-base balance, serum protein concentration and packed cell volume between infants delivered vaginally and by cesarian section were noted. The explanation of these differences is not known.

### Acknowledgement

The authors express their appreciation to the nursing and resident staffs of the Department of Obstetrics for their cooperation during this study and to Dr. John C. Ullery for making the study possible.

### References

1. APGAR, V.: A proposal for a new method of evaluation of the newborn infant. *Curr Res Anesth Analg*, 32: 260, 1953.
2. ASTRUP, P., JØRGENSEN, K., ANDERSEN, O. S. and ENGEL, K.: The Acid-base metabolism. A new approach. *The Lancet* (in press).
3. BASINKI, D. H.: Reported at meeting of Amer Ass Clin Chemists, Cleveland, Ohio, Aug. 29, 1959.
4. BATES, G. B. and OLIVER, T. K., Jr.: A micromodification of the bubble method for the direct determination of blood-gas tensions. *J Lab Clin Med*, (submitted).
5. BEER, R., BARTELS, H. and RACZKOWSKI, H. A.: Sauerstoffdissociationskurve des fetalen Blutes und der Gasaustausch in der menschlichen Placenta. *Arch Ges Physiol*, 260: 306, 1955.
6. BOUTERLINE-YOUNG, H. and BOUTERLINE-YOUNG, E.: Alveolar carbon dioxide levels in pregnant, parturient and lactating subjects. *J Obstet Gynaec Brit Emp*, 63: 509, 1956.
7. BRADLEY, A. F., STUFFEL, M. and SEVERINGHAUS, J. W.: Effect of temperature on  $\text{pCO}_2$  and  $\text{pO}_2$  of blood in vitro. *J Appl Physiol*, 9: 201, 1956.

8. COOK, C. D., CHERRY, R. B., O'BRIEN, D., KARLBERG, P. and SMITH, C. A.: Studies of respiratory physiology in the newborn infant. I. Observations on normal premature and full-term infants. *J Clin Invest*, 34: 975, 1955.
9. DAVENPORT, H. W.: ABC of Acid-Base Chemistry. The University of Chicago Press, 3rd ed., 1950.
10. DAWES, G. S.: Changes in the circulation at birth and the effects of asphyxia, in Recent Advances in Pediatrics, Intersciences Publishers, Ltd, London, 1958, p 1.
11. EARL, D. P., BAKWIN, H. and HIRSCH, D.: The plasma potassium level in the newborn, *Proc Soc Exp Biol Med*, 76: 756, 1951.
12. ELDRIDGE, F. L. and HULTGREN, H. N.: Physiologic closure of the ductus arteriosus in the newborn infant. *J Clin Invest*, 34: 987, 1955.
13. GIEBISCH, G., BERGER, L. and PITTS, R. A.: The external response to acute acid-base disturbance of respiratory origin. *J Clin Invest*, 39: 231, 1955.
14. GORNALL, A. G., BURDAWILL, C. J. and DAVID, M. M.: Determinations of serum proteins by means of the biuret reaction. *J Biol Chem*, 177: 751, 1949.
15. GRAHAM, B. D. and WILSON, J. C.: Chemical control of respiration in newborn infants. *Amer J Dis Child*, 87: 287, 1954.
16. GRAHAM, B. D., WILSON, J. C., TSAO, M. U., BAUMAN, M. L. and BROWN, S.: Development of neonatal electrolyte homeostasis. *Pediatrics*, 8: 68, 1951.
17. — in Adaptation to Extrauterine Life, Report of the thirtyfirst Ross Conference on Pediatric Research, Columbus, Ross Laboratories, 1959, p. 57.
18. JAMES, L. S.: idem p. 28.
19. — idem p. 70.
20. JAMES, L. S., WEISBROT, I. M., PRINCE, C. E., HOLADAY, D. A. and AFGAR, V.: The acid-base status of human infants in relation to birth asphyxia and the onset of respiration. *J Pediat*, 53: 399, 1958.
21. — Acidosis of the newborn and its relation to birth asphyxia. *Acta Paediat*, 49: 17, 1960, Suppl. 122.
22. KARLBERG, P.: The adaptive changes in the immediate postnatal period with particular reference to respiration. *J Pediat*, 56: 585, 1960.
23. LIND, J.: Changes in the circulation and lungs at birth. *Acta Paediat*, 49: 39, 1960. Suppl. 122.
24. MC CLURE, J. H.: Newborn blood oxygen. A method of increasing the partial pressure of oxygen in the blood of the newborn infant. *Obstet Gynec*, 11: 696, 1958.
25. NATELSON, S.: Routine use of ultramicro methods in the clinical laboratory. *Amer J Clin Path*, 21: 1153, 1951.
26. OARD, H. C. and PETERS, J. P.: The concentration of acid and base in the serum in normal pregnancy. *J Biol Chem*, 81: 9, 1929.
27. PINCUS, J. B., GITTLEMAN, I. F., SAITO, M. and SOBEL, A. E.: A study of plasma values of sodium, potassium, chloride, carbon dioxide, tension, sugar, urea and the protein base binding power, pH and hematocrit in prematures on the first day of life. *Pediatrics*, 18: 39, 1956.
28. PREC, K. J. and CASSELS, D. E.: Oxymeter studies in newborn infants during crying. *Pediatrics*, 9: 756, 1952.
29. REARDON, H.: in Adaptation to Extrauterine Life. Report of the thirtyfirst Ross Conference on Pediatric Research, Columbus, Ross Laboratories. 1959, p. 79.
30. REARDON, H., BAUMANN, M. L. and HADDAD, E. J.: Chemical stimuli of respiration in the early neonatal period. *J Pediat*, 57: 151, 1960.
31. ROTH, G., SJÖSTEDT, S. and CALIGARA, F.: The "in vivo" foetal oxygen dissociation curve. *Biol Neonat*, 1: 61, 1959.
32. SCRIBNER, B. H., FREMONT-SMITH, K., BURNELL, J. M.: The effect of acute respiratory acidosis on the internal equilibrium of potassium. *J Clin Invest*, 39: 1276, 1955.
33. — Potassium intoxication during and immediately following respiratory acidosis. *J Clin Invest*, 33: 965, 1959.
34. SCHALES, O. and SCHALES, S. S.: Simple and accurate method for determination of chloride in biological fluids. *J Biol Chem*, 140: 879, 1941.
35. SEVERINGHAUS, J. W., STUFFEL, M. and BRADLEY, A. F.: Variations of serum carbonic acid pK with pH and temperature. *J Appl Physiol*, 9: 197, 1956.
36. — Oxyhemoglobin dissociation curve correction for temperature and pH variation in human blood. *J Appl Physiol*, 12: 785, 1958.
37. SIMMONS, D. H. and AVEDON, M.: Acid-base alterations and plasma potassium concentration. *Amer J Physiol*, 197: 319, 1959.
38. SINGER, R. B. and HASTINGS, A. B.: An improved clinical method for the estimation of disturbances of the acid-base balance of human blood. *Medicine (Balt)*, 27: 223, 1948.
39. SISSON, T. R. C. and WHALEN, L. E.: The blood volume in infants. III. Alterations in the first hour after birth. *J Pediat*, 56: 43, 1960.

40. SJÖSTEDT, S., ROTH, G. and CALIGARA, F.: The oxygen tension in the cord blood after normal delivery. *Acta Obstet Gynec Scand*, 39: 34, 1960.
41. STAHLMAN, M. T.: Pulmonary ventilation and diffusion in the human newborn infant. *J Clin Invest*, 36: 1081, 1957.
42. STERN, L. and LIND, J.: Cardiovascular Disease: Perinatal circulation in *Ann Rev Med*, 11: 113, 1960.
43. USHER, R.: The respiratory distress syndrome of prematurity. I. Changes in potassium in the serum and the electrocardiogram and effects of therapy. *Pediatrics*, 24: 562, 1959.
44. — presented at IX International Congress of Pediatrics, Montreal, Canada, July 1959.
45. WEISBROT, I. M., JAMES, L. S., PRINCE, C. E., HOLADAY, D. A. and APGAR, V.: Acid-base homeostasis of the newborn infant during the first 24 hours of life. *J Pediat*, 52: 395, 1958.
46. WIDDOWSON, E. M. and McCANCE, R. A.: The effect of development on the composition of the serum and extracellular fluids. *Clin Sci*, 15: 361, 1956.
47. WHEELER, W. E., BATES, G. D. and OLIVER, T. K., Jr.: A micromethod for the determination of pH (To be published).

Submitted Nov. 18, 1960

Accepted Nov. 25, 1960

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## The Cellular Excretion in the Urine of Normal Newborn Infants

by KJELL AAS

The method of estimating the excretion of cellular elements in the urine as suggested by Addis (1) has been widely used since 1926, and his standards for adults are constantly referred to in the medical literature. Very few comprehensive studies of the urinary sediment from children have been published. In 1932 Lyttle first reported the Addis count in normal children (10). His study included 74 children between the ages of 4 and 12 years, and his method corresponded closely with that used by Addis with some modification of the strict fluid restriction. His results were in agreement with the standards for adults (Table 2). Counts of red cells and casts in the urine from 202 children were reported by Snoke (15), but he did not count epithelial or white cells. A study by Giles (4) included only 20 children. From the information given in her paper that "in any case where bedwetting was suspected, the urine was discarded", one may assume that the children observed were beyond infancy. Although none of the studies mentioned here include the urine of neonates, their results are referred to as standards for infants as well as adults in several publications including textbooks of urology.

Stansfeld & Webb (17), James (7) and

others found the Addis count cumbersome to perform and impracticable for everyday use, and most workers in this field believe that counting low or high power fields is too inaccurate and crude to be accepted. Instead it has been advocated that the count should be done on uncentrifuged urine in a white-cell-counting chamber and the number of cells per cubic millimetre recorded (7, 17). By this method Stansfeld & Webb studied the urine of 632 normal children; 127 boys and 75 girls were under one year of age, but very few, if any, were neonates (16), which also may be assumed from the statement "insignificant numbers of epithelial cells were present ...". In neonates a fairly high proportion of renal epithelial cells is found in the urine sediment especially in the very first days of life. In two studies (3, 11) it was found that the urine from male and female infants usually contained less than 10 cells per  $\text{mm}^3$  and very rarely more than 50 cells per  $\text{mm}^3$ , whereas in older girls the upper limit of normal was set at 100 cells per  $\text{mm}^3$  and 500 cells per  $\text{mm}^3$ , respectively. James (7), in a study of 50 male and 50 female infants between the ages of 6 and 10 days, found that the urine usually contained less than 20 pus cells per  $\text{mm}^3$ .



No exact information is available regarding the normal cell excretion in the first days of life. Nevertheless such information is imperative for the discussion of urinary findings in pathological conditions in neonates. It is the purpose of this paper to discuss observations on the cell excretion in the urine of normal newborn infants. Specimens of preformed urine collected during or within minutes after delivery as well as specimens obtained during the first week of life were studied.

### Material and Method

Most urine samples were obtained in the Obstetric Department of the University Clinic, Rikshospitalet. A few were obtained in a general practice. Only infants of healthy mothers with uncomplicated pregnancies and uneventful deliveries were included. Infants with respiratory distress of the slightest degree or with any known pathological trait were excluded. The neonates were examined by a pediatrician specially associated with the obstetric department, as well as by the author. The infants were fed according to modern principles of neonatal care (12). Only those with a normal weight curve and without signs of unphysiological dehydration were included in the study. A total of 380 specimens obtained at one or two day intervals from 114 male and 27 female infants were studied. The preponderance of males reflects the convenience of sampling.

A proportion of infants void immediately at birth, and alert midwives were able to collect some samples in the air. These specimens were immediately stained for microscopic examination. Many infants void when unclothed, or when the suprapubic and perineal regions are being cleaned. These specimens were easily collected for examination. Most samples, however, were collected over 12- and 24-hour periods.

In the boys a plastic colostomy bag with a central hole surrounded by adhesive was fitted around the penis after thorough cleansing of the skin and prepuce. To avoid artificial bleeding the prepuce was not withdrawn. In female infants a soft funnel made from one-half of a rubber ear syringe was fitted around the entire vulva and labia majoris after cleansing. The funnel was held in place by adhesive bands fixed to the abdomen and the gluteae, exerting a firm pressure especially on the perineum. It was made certain that the anus was entirely free and outside this elastic funnel. The plastic bag was then fixed around the outer opening of the funnel. When only examination of the sediment was to be performed a few drops of stain were placed in the bag before fixing in position, otherwise no stain was added at that time. The stain described by Sternheimer & Malbin (18) was used. A few drops of formaldehyde were also added to secure good fixation of the cellular elements.

The plastic bag was in position for a few hours, 12 hours or 24 hours according to purpose. The bag was emptied at intervals without being removed by cutting a hole which was sealed afterwards with adhesive bands.

Examination of all urines included pH, specific gravity, protein and sugar in addition to examination of the sediment. In some infants additional urinary studies (electrolytes and amino acid excretion, as well as capillary blood analyses of NPN, creatinine, hematocrit, electrolytes and CRP) were performed. These results will subsequently be published (5). Catheterization of female infants was not performed. It was felt that the use of the catheter should be restricted to more imperative occasions (2). The risk of contamination of the urine by leucocytes from the vulva is relatively low in newborn female infants (4), although it is significant in some cases. Two girls with slight physiological vaginal bleeding on the 5th and 8th day after birth were excluded from the present material. Hematuria which can appear in normal boys was not observed in this study. Because the

sampling from female infants was complicated by leaking at the perineal rim only males were used for timed urine collections.

The urine sample was thoroughly mixed, following which 5 or 10 ml were centrifuged for 5 minutes at 2000 r.p.m. The supernatant was decanted leaving a final volume of 0.1 the initial volume. One or two drops of stain were then added and shaken. The specimen was then allowed to stand for 15-30 minutes before examination. If kept longer the stained specimen was stored in a refrigerator at  $+4^{\circ}\text{C}$ . The sediment was re-mixed, then counted in a white-cell-counting chamber under both low and high power as well as by phase contrast in most instances.

The stain was used to ensure good fixation of the organic sediment, and also to distinguish the cell structures. It was especially valuable in the differentiation between leucocytes and renal epithelial cells (non-squamous epithelial cells). This differentiation is difficult and very often an impossible task even for experienced investigators (9). In this study differentiation was attempted by tabulating the cells in three categories: typical non-squamous epithelial cells of renal type, typical leucocytes, and cells of more uncertain structure. Often the last category was predominant. The total of the three categories was tabulated; epithelial cells other than those of assumed renal origin were not counted. All counts were performed two or three times and the mean tabulated.

## Results

The results obtained from examination of the specimens from the 141 infants are illustrated in Fig. 1. One girl was omitted from the study because of a leucocyte count which exceeded 1500 cells per  $\text{mm}^3$ . A catheterized specimen contained a number of cells similar to that found in most of the other infants and she was subsequently found to have a purulent vaginitis. The sediment counts from the

other female infants did not differ appreciably from the males. Vaginal epithelial cells were present, but the leucocyte counts were not among the highest ones in this series of neonates. Accordingly sex differentiation was not pointed out in the figure. The average number of cells excreted in the preformed urines was 10 per  $\text{mm}^3$  and the highest number was 30 per  $\text{mm}^3$ . One specimen was devoid of leucocytes and epithelial cells, although two erythrocytes were present. Most specimens contained 0-4 erythrocytes per  $\text{mm}^3$ . Casts were found in six cases. In one specimen two casts were noted, while the other five contained only one cast per  $\text{mm}^3$ . The casts were of the hyaline type with or without epithelial cells or cell debris incorporated. The specific gravity, pH, albumin and sugar content were similar to those found by others in preformed urines (14).

The great variance between infants in the combined counts of white and epithelial cells is clearly illustrated in Fig. 1. Both the average for each day and the normal range are tabulated in Table 1. No specimen was free of cells, in contrast to what has been observed by others (1, 4, 10). This is probably because the examination was performed on centrifuged urine in this study. Centrifuging here gives a tenfold greater chance of discovering cells in the 0-10 cell per  $\text{mm}^3$  range than does examination of uncentrifuged urine. For the first few days of life renal epithelial cells outnumbered the leucocytes in most infants. At the end of the first week the leucocytes tended to equal or outnumber the epithelial cells. The proportion between them could not be calculated because the uncertain group, "leucocytes or epithelial

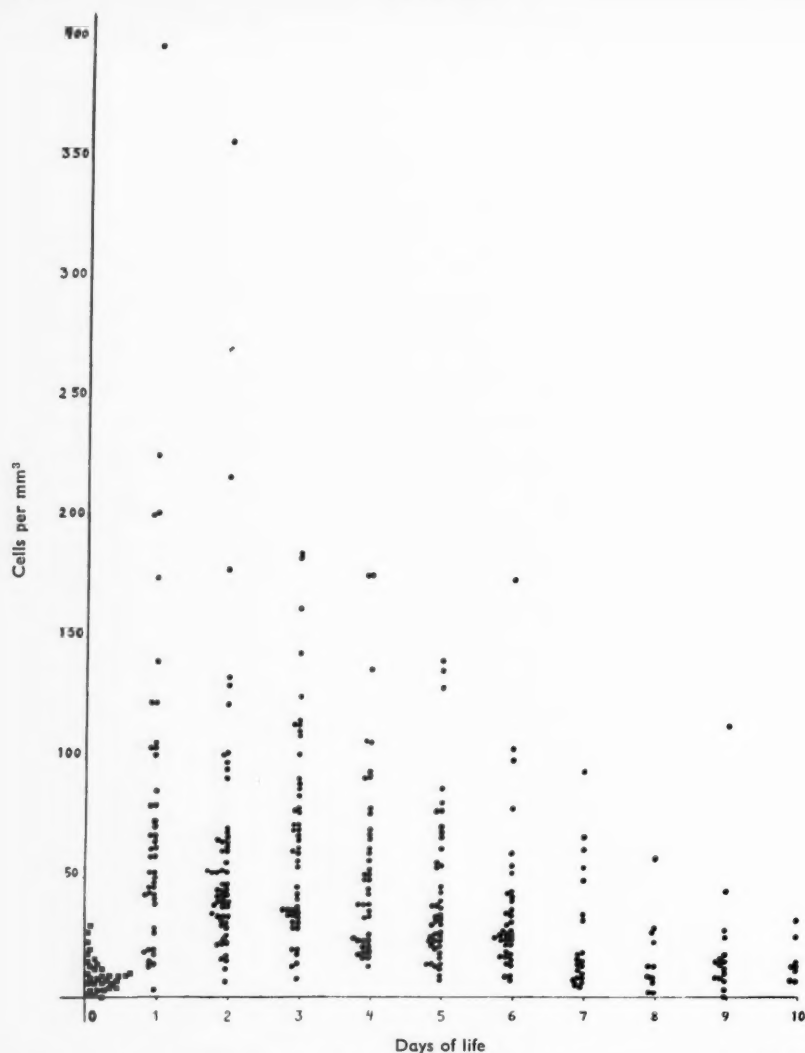


Fig. 1. Sum of epithelial cells and leucocytes excreted per cubic millimetre. ■ voided at birth, preformed urine, ● other specimens.

cells" was dominant in a majority of counts. An example of these variations is illustrated in Table 3. Great variability in counts from specimen to specimen was observed in each individual, and is

magnified when the cell excretion is expressed as an Addis count.

The objections to the Addis count which have been made by several authors (6, 7, 17), also apply when the method is

TABLE 1. *Total leucocytes and non-squamous epithelial cells excreted per cubic millimetre of urine.*

Day	Preformed urine	1	2	3	4	5	6	7
Average	10	78	65	67	56	43	37	26
Range	0-30	3-397	7-357	8-185	13-176	7-140	7-174	4-94
Number of specimens	33	45	59	50	42	45	37	21

adjusted for infants. Voiding is uncontrolled and appears at irregular intervals; furthermore, it is uncertain whether the bladder is completely emptied or not at each voiding. In addition the urine output is very variable. Some infants retain the urine in the bladder for long periods making considerable intravesicular cell lysis possible. A proportion of infants do not void at all in the first 48-72 hours of extrauterine life (19). Timed sampling is impossible without repeated catheterization. This difficulty was exemplified in one infant, who, during the 12-hour sampling period, voided only 4 ml. Just as the bag was removed and he was being washed he

urinated 21 ml. The few minutes added to the sampling period would increase the Addis count six fold! In order to compare the sediment counts in the present study with those done by others, the Addis count has been calculated for infants from whom the collection procedure was optimal (Table 2).

The daily urine volumes computed from 12-hour periods, and partly by sampling for 24 hours are found in Table 4. When the results are compared with those of Thomson (19), a remarkable conformity is found. With the normal range in mind, it is understood that computing the 12-hour secretion of cells from theoretical volumes

TABLE 2. *The range and the average Addis sediment counts of cells and casts in the urine from newborn infants compared to those of normal children and adults.*

R = range, A = average.

Series		Leucocytes and renal epithelial cells	Erythrocytes	Casts
Addis (adults)	R	32,000-1,835,000	0-425,000	0-4270
	A	322,000	65,750	1040
Lyttle (74 children)	R	9000-2,822,000	0-120,000	0-12,916
	A	322,184	15,181	1085
Snoko (202 children)	R		0-800,000	0-29,000
	A		81,000	1230
Giles (20 children)	R	9000-714,000	0-438,000	0-6350
	A	329,000	61,325	1175
Author (119 specimens first 6 days)	R	42,000-13,500,000	0-630,000	0-440,000
	A	1,865,529	90,219	12,852

TABLE 3. *Serial sediment counts from one male infant.*

Cell type	Day				
	0	2	3	5	7
Leucocyte	1	30	4	9	6
Non-squamous (renal) epithelial	3	48	27	6	3
Uncertain	5	13	20	7	3
Total	9	91	51	22	12

TABLE 4. *Average and range of daily volume of urine. Computed from 12-hour and 24-hour specimens. The corresponding figures of Thomson (19) in brackets.*

Day	Volume ml	Range ml	Number of infants
1	16.8 (19.5)	0-68 (0-68)	32 (35)
2	34.1 (20.6)	0-84 (0-82)	30 (29)
3	63.8 (36.0)	0-71 (0-96)	27 (26)
4	86.4 (64.8)	14-160 (5-180)	27 (26)
5	125.6 (103.3)	44-216 (1-217)	17 (23)
6	134.8 (124.5)	14 <sup>a</sup> -260 (42-268)	16 (19)

<sup>a</sup> 12-hour specimen; the next lowest was 40 ml in 24 hours.

as done by Snoke in his study (15), will give erroneous results in neonates. Accordingly, it is not attempted in the present paper.

Of the 380 specimens examined, 145 contained erythrocytes. Red blood cells were most abundant during the first days of life and subsequently declined in number. After the first week they were sparse. In dilute and alkaline urines erythrocytes disintegrate very quickly, making the examination of the newly voided urine imperative unless fixation is used. This also applies to casts. Casts were found in almost every specimen. They were mostly of the granular type, or hyaline with epithelial cells or cell debris incorporated. Occasional epithelial casts were seen. In a few casts one or two typical leucocytes were found incorporated side by side with renal epithelial cells. Three infants with

such casts were followed for 8-14 weeks. No sign of renal disease or urinary infection was found, the infants thrived perfectly and all follow-up urine specimens have been normal. The Addis counts for erythrocytes and casts in the newborn infants are compared with the similar figures published by others (4, 10, 15) in Table 2.

The method of counting the cells in a hemocytometer was compared with the more conservative method of judging the cell excretion by high-power field microscopy (Table 5). In the present study this comparison applies to centrifuged urine. Conformity is found with the results published by Stansfeld & Webb (17), who studied uncentrifuged urine.

Some specimens were studied to determine the degree of cell lysis which occurred when the urine remained in the plastic

TABLE 5. *Comparison of cell excretion expressed in cells per mm<sup>3</sup> to high-power field microscopy.*

Cells per mm <sup>3</sup>	Cells per h.p.f.
400—	10-50 (numerous, heaps)
200-400	8-30
100-200	5-20
50-100	2-10
20- 50	0- 1, occasional
0- 20	None, occasional

TABLE 6. *Number of cells remaining intact after 3, 6, 12 and 24 hours. Unstained specimens at body temperature. Stained specimens at +4°C.*

Specimen	S = stained U = unstained	Hours				
		0	3	6	12	24
A.N.	S	22		21	23	20
	U	22	17	18	15	9
J.T.	S	24	27	23		23
	U	24	23	19	14	10
E.E.	S	339	353		299	320
	U	339	311		254	173
M.P.	S	62		60	65	56
	U	62		55	38	21
E.P.	S	94	91	91	108	99
	U	94		70	64	57

bag for some time. Specimens were obtained immediately after voiding. Five ml were examined and the remaining urine was placed in a plastic bag close to the infant beneath the diaper. Other specimens were stored at 37°C. At intervals of 3, 6, 12 and 24 hours 5 ml aliquots were examined. The progress of cell lysis after staining and storing in a refrigerator at +4°C was studied in a similar way. The results of the lysis study are found in Table 6. The cells undergo a very significant lysis when kept in the plastic bag at body temperature. A similar process of

cell lysis also probably occurs when the urine is retained in the bladder. Disintegration progresses were fastest in erythrocytes and hyaline casts. The epithelial cells were the most resistant. The lysis was most prominent in dilute urines and at a high pH. The degree of lysis in unstained specimens corresponded fairly well with that found by Houghton and Spears in urine from adults (6). The cells were not influenced much by lysis when the urine was stained as described and stored in a refrigerator. This is a practical point for the clinician who is not always able to

study the urine specimen under the microscope immediately after the specimen is produced. For studies of urine sediment only, a few drops of stain may be placed in the bag before it is positioned.

### Discussion

It has long been known that the urine of the normal newborn infant contains a higher proportion of cells and casts than that found in older children and adults (3, 11). This is confirmed by the present study. It was also observed that the sediment count in normal neonates often exceeds the limits for children and adults established by earlier investigators. This is true for erythrocytes and casts and even more so for leucocytes and non-squamous epithelial cells assumedly of renal origin. In the first days after birth the infant's urine is rich in non-squamous epithelial cells which are believed to originate from the lining of distal tubules and collecting ducts. The abundance of such cells in the urine is probably a result of a rapid growth of the epithelial lining with a lively replacement of cells. The presence of numerous leucocytes suggests that blood cells enter the lumen through the epithelial lining, possibly through small transient defects in this lining under normal conditions. The demonstration of leucocytes in casts from some neonates proves that at least some leucocytes enter the urine proximal to the collecting tubes, as these casts were not of the broad type. In older children and adults the presence of leucocytes in casts is suggestive of a pathological condition (9, 13). In neonates, however, the passage of leucocytes through the epithelial lining is probably made pos-

sible by the exfoliation and renewal of cells at this age.

It is now generally accepted that sediment counts of more than 100 cells per  $\text{mm}^3$  are highly suggestive of renal disease. Counts of 20–100 cells per  $\text{mm}^3$  induce suspicion of a pathological condition and necessitate repeated examinations. Counts of 20 cells per  $\text{mm}^3$  or less are considered normal (6, 7, 16). These limits do not apply to the sediment counts in the urines of neonates. In the present study more than 20 cells per  $\text{mm}^3$  were found in the majority of specimens examined. One hundred and thirty-nine specimens contained more than 50 cells per  $\text{mm}^3$ , and of the 380 specimens examined 43 contained more than 100 cells per  $\text{mm}^3$ . Only seven counts exceeded 200 cells per  $\text{mm}^3$ . The infants with extraordinarily high sediment counts were followed closely for 10 to 15 days. All counts fell to average values during this time and it was not possible to demonstrate pathological traits in any of the infants. There is evidence, however, that both intrauterine asphyxia and perinatal asphyxia may induce transient renal damage with increased cell excretion into the urine (5, 8). It is possible that a milder degree of hypoxia of some duration has a similar effect on the fetal kidney. Conditions with a moderate fetal hypoxia may be concealed during the pregnancy. The highest sediment counts in the present study suggest that some intrauterine renal damage occurred in these infants. Uric acid infarctions might possibly play a part, although no correlation could be found between the amounts of organic and inorganic sediment in the present study.

The sediment counts are usually at the highest levels in the urines formed shortly



after birth. There is a fast decline in number of cells and casts excreted during the first 6 to 10 days of life, and after this period the number of cells found is similar to that reported in studies of older infants and children. Normal birth is probably accompanied by some shock reaction in the infant's organs. Theoretically transient hypoxemia of the renal tissue caused by the birth process may be an important mechanism in the high sediment counts. In the present study there was no relation between the sediment counts and the duration of labour. No comparisons of the oxygen saturation of the infant's blood during and after delivery have been performed and thus no certain conclusion can be reached in this regard. It is equally tempting to suggest that the increased cell excretion is more the result of rapid tissue renewal in the tubule linings rather than changes induced by the "shock" of being born. The low content of cells found in the preformed urines is probably the result of intravesicular cell lysis in the diluted fetal urine.

Three infants delivered by cesarean section before labour had started were followed, although omitted from the material of normal infants. The sediment counts of these infants were all close to the average found in the present material.

The ranges of the normal Addis count for children and adults by earlier investigators (1, 4, 10, 14) are too narrow for newborn infants. In addition to being cumbersome to perform, the Addis count suffers from great inaccuracies due to sampling difficulties in infants and is not to be relied upon. The method of expressing the sediment excretion in cells per cubic millimetre is more reliable. A combination of the two gives the best information of the sediment quantities. The standards given for newborn infants in the present study will provide a better foundation for comparison of such sediments in health and disease.

### Summary

Urine sediment counts were made in 380 specimens collected from 114 male and 27 female infants during the first 10 days after birth. In some the Addis counts were calculated. This method is considered inaccurate and erroneous in infants, however, and it is advocated that the findings should be expressed in cells per cubic millimetre. The counts found in the normal newborn infants are tabulated and the results are discussed. The range of normal sediment counts found exceeds that set for older children and adults in earlier publications.

### References

1. ADDIS, T.: A clinical classification of Bright's disease. *JAMA*, 85: 163, 1925.
2. BEESON, P. B.: The case against the catheter. *Amer J Med*, 24: 1, 1958.
3. CRAIG, W. S.: Urinary disorders occurring in the neonatal period. *Arch Dis Child*, 10: 337, 1935.
4. GILES, M. D.: The Addis count in the prognosis of acute nephritis in childhood. *Arch Dis Child*, 22: 232, 1947.
5. HALVORSEN, S. and AAS, K.: Observations of the urine of asphyxiated and post-mature infants (to be published).
6. HOUGHTON, B. J. and PEARS, M. A.: Cell excretion in normal urine. *Brit Med J*, 1: 622, 1957.
7. JAMES, U.: Urinary infection in the newborn. *Lancet*, 1: 1001, 1959.
8. JONSSON, B.: Lower nephron nephrosis in asphyxia neonatorum. *Acta Pædiat*, 40: 401, 1951.
9. LIPPMAN, R. W.: Urine and the Urinary

- Sediment. A Practical Manual and Atlas, p. 25. C. C. Thomas, Springfield 1957.
10. LITTLE, J.: The Addis sediment count in normal children. *J Clin Invest*, 12: 87, 1933.
  11. REUSS, A. R. VON: Die Krankheiten des Neugeborenen, S. 26. Julius Springer, Berlin 1914.
  12. SALOMENSEN, L.: Propedeutisk pediatri, p. 149. J. Grundt Tanum, Oslo 1952.
  13. SCHREINER, G. E.: The identification and clinical significance of casts. *Arch Int Med*, 99: 356, 1957.
  14. SMITH, C. A.: The Physiology of the New-born Infant, p. 329. C. C. Thomas, Springfield, Ill. 1959.
  15. SNOKE, A. W.: The normal Addis sediment count in children. *J Pediat*, 12: 473, 1938.
  16. STANSFELD, J. M.: Personal communication 1960.
  17. STANSFELD, J. M. and WEBB, J. K. (J.): Observations on pyuria in children. *Arch Dis Child*, 38: 386, 1953.
  18. STERNHEIMER, R. and MALBIN, B.: Clinical recognition of pyelonephritis, with new stain for urinary sediments. *Amer J Med*, 11: 312, 1951.
  19. THOMSON, J.: Observations on the urine of the new-born infant. *Arch Dis Child*, 19: 169, 1944.

Submitted Nov. 8, 1960

Accepted Nov. 17, 1960

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## Serum-total and L(+)-Tartaric-acid-inhibited Acid-phosphatase Activity in Various Diseases in Childhood<sup>1</sup>

by ZVI LARON and AVIVAH KOWADLO

In 1937 Gutman & Gutman demonstrated the presence of acid phosphatase in human serum and found increased activity in patients with metastasizing prostatic carcinoma. Since then occasional increased activity has been reported in adults in other diseases, such as Paget's disease (13), hyperparathyroidism (16), osteoporosis (8), liver disease (3, 6), Gaucher's disease (23) and gonadal dysgenesis (7). In children in whom the normal serum acid-phosphatase activity is higher than in adults (18), increased serum activity of this enzyme has been reported in rare instances of osteopetrosis (24), osteogenesis imperfecta (10, 21), Niemann-Pick's disease (14) and more recently in Gaucher's disease (5).

In the present investigation serum-total and tartaric-acid-inhibited acid-phosphatase activities have been measured in children suffering from a variety of diseases with the aim of defining under which conditions the activity of these enzymes is altered in childhood.

### Subjects and Methods

Children from the Pediatric Department and Pediatric Metabolic and Endocrine

Clinic were studied. Blood was taken from the antecubital vein and the serum was separated within one to two hours. Hemolyzed samples were discarded. Acid-phosphatase activity was determined immediately or after storage in the deep freeze for periods not longer than 3 days. According to our experience, storage did not alter the enzyme activity.

Serum-total acid phosphatase (TAP) was determined by the method of King (15), slightly modified as described previously (18). L(+)-tartaric-acid-inhibited acid phosphatase (TIAP) was determined according to Fishman & Lerner (9). The mean normal values obtained in our laboratory for TAP are: age 1 year,  $5.23 \text{ KAU} \pm 1.26 \text{ (SD)}$ ; 2-8 years,  $4.63 \text{ KAU} \pm 0.93 \text{ (SD)}$ ; 8-13 years,  $4.92 \text{ KAU} \pm 0.98 \text{ (SD)}$ ; TIAP for all ages,  $0.21 \text{ KAU} \pm 0.28 \text{ (SD)}$ . Alkaline phosphatase was measured in Bodansky units (2).

### Results

The overall results of the TAP and TIAP measurements obtained are graphically shown in Figs. 1 and 2 and analysed for the various diseases in Tables 1-2.

#### *Rheumatic fever*

Twenty-four children suffering from acute rheumatic fever were studied either before or during the first days of treatment (Table 1). With one exception the laboratory data in all cases pointed to

<sup>1</sup> This study was supported by a grant from the General Federation of Labour.

TABLE 1. Serum acid-phosphatase activity in children with acute rheumatic fever.

Patient	Age, years	Sex	Total acid-phosphatase, KA units <sup>a</sup>	Tartaric-acid-inhibited acid-phosphatase, KA units <sup>b</sup>	ESR 1st hour	CRP	Weltman
1. C.B.	6	F	5.6	0	46	—	—
2. K.B.	6	F	3.6	0.8	115	—	1
3. J.M.	7	M	5.5	1.2	98	—	3
4. A.B.	8	M	7.3	0.9	—	—	—
5. I.K.	8	M	6.7	1.2	30	—	5
6. M.C.	8	M	5.9	0	138	—	1
7. M.M.	8	M	4.5	0.5	—	—	—
8. R.M.	8	M	6.5	0.3	45	0	—
9. Y.B.	9	M	5.7	1.5	60	3+	1
10. H.S.	9	F	2.9	0.5	118	4+	4
11. T.A.	10	F	7.3	1.5	120	2+	1
12. R.G.	10	F	3.4	0.2	138	—	0
13. E.F.	10	F	3.9	0	35	0	5
14. I.T.	10	M	5.7	2.3	102	4+	0
15. D.S.	10	M	6.4	2.0	130	—	0
16. E.S.	10	M	4.9	3.1	115	4+	1
17. B.D.	11	M	3.4	0.2	—	—	—
18. Y.Z.	11	M	4.9	0	66	3+	3
19. G.R.	11	M	7.4	2.7	3	1+	—
20. C.M.	11	M	5.4	0.4	—	—	—
21. Y.V.	11	M	7.0	1.8	95	—	1
22. G.S.	11	F	5.3	1.3	94	—	—
23. E.S.	12	F	7.8	2.0	100	—	—
24. D.P.	12	M	7.1	—	63	3+	—

<sup>a</sup> Mean 5.6,  $\pm 1.66$  (SD).<sup>b</sup> Mean 1.1,  $\pm 0.94$  (SD).

rheumatic activity, as indicated by the ESR, and Weltman reactions.

In 10 children (41%) the activity of TAP was within the range of the normal mean  $\pm 1$  SD; in most of them, however, it was higher than the mean. In 10 additional children the TAP activity was more than 1 SD from the mean; and in five instances (21%), more than 2 SD higher. In four cases (16.5%) the activity was more than 1 SD below the mean. The mean TAP activity in rheumatic fever was  $5.6 \text{ KAU} \pm 1.66$ . Compared with the normal mean in the age groups 6–13 years, the difference was not significant ( $t = 1.93$ ;  $P < 0.1$ ).

TIAP activity was measured in 23 instances. In 10 children (43%) it was

within the range of the normal mean  $\pm 1$  SD. In 13 children (56%) the TIAP activity was more than 2 SD higher than the normal mean. The mean activity was  $1.1 \text{ KAU} \pm 0.94$  and its difference from the normal mean was statistically significant ( $t = 4.2$ ;  $P < 0.001$ ).

The elevation of TAP was not accompanied by elevation of TIAP in all the cases; in a few instances increased activity of TIAP was found when the activity of the TAP was within the normal range.

In seven children repeated serum analyses were made during and after treatment; in six of them both the TAP and TIAP activities decreased one to two weeks after institution of therapy.

TABLE 2. Serum acid-phosphatase activity in children with bone disease.

Patient	Age years	Sex	Diagnosis	Total acid- phosphatase, KA units	Tartaric-acid- inhibited acid- phosphatase, KA units	Alkaline phosphatase, Bodanky units
1. C.B.	3/12	M	Rickets	11.3	1.1	21.4
2. A.C.	3/12	M	Rickets	4.8	0.3	—
3. Z.C.	4/12	M	Rickets	5.1	0.2	29.6
4. S.G.	4/12	F	Rickets	3.9	0.3	18.4
5. T.A.	5/12	F	Rickets	9.7	—	24
6. I.S.	5/12	M	Rickets	5	0.1	14.8
7. I.G.	7/12	M	Rickets	3.3	0	15
8. S.J.	10/12	M	Rickets (healed)	5.5	—	18.7
9. A.L.	11/12	M	Rickets	2.5	—	24.6
10. M.N.	1/365	F	Hypocalcemia	5.1	0	10.8
11. M.S.	3/12	F	Osteogenesis imp.	7.2	2.4	4
12. D.B.	14/12	M	Familial dys- chondroplasia	5	—	—
13. B.P.	6	F	Multiple mal- formations of spine	6.0	0	—
14. M.H.	8	F	Dyschondro- plasia	5.5	1.8	9
15. B.G.	9	M	Bone cyst	3.1	—	—
16. C.O.	10	F	Mult.cong. osseous mal- formations	3.5	—	5
17. N.S.	11	M	Osteophytes	4.8	—	13.6
				5.4	0.3	—

*Bone diseases*

In nine infants with rickets (Table 2) serum TAP activity was markedly increased in two and lower than 1 SD below the mean in three cases, being within the range of mean  $\pm$  1 SD in the remaining. TIAP determined in six infants was abnormally elevated in only one instance.

Serum alkaline-phosphatase activity was elevated in six of the children, but there was no correlation with any of the acid phosphatases. Among other bone diseases, one 3-month-old infant with osteogenesis imperfecta exhibited increased activity of both TAP and TIAP.

TAP as well as TIAP have been determined also in various other diseases as shown in the Figures 1 and 2.

*Discussion*

Acid phosphatases are widely distributed enzymes (25) but our knowledge is scant concerning their metabolic role in normal and pathological states (26).

Using different substrates and inhibitors, an attempt was made to differentiate several acid phosphatases: the prostatic phosphatase is inhibited by L(+)-tartaric-acid (21); erythrocyte acid phosphatase is inhibited by formaldehyde (6),

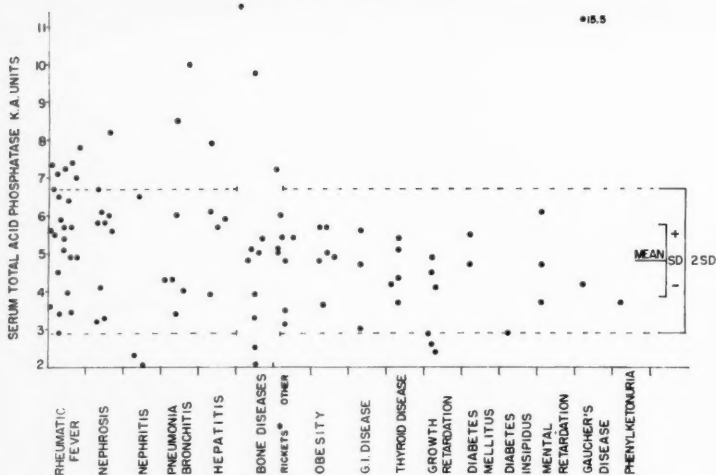


Fig. 1. Serum-total acid-phosphatase activity in a variety of diseases in childhood. The normal mean is applicable to all groups besides "rickets". Since this disease occurs in infants under the age of one year, the normal mean is slightly higher.

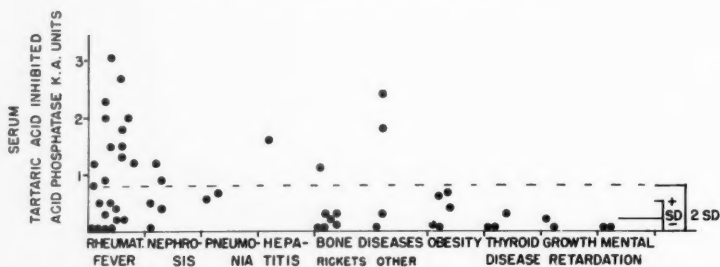


Fig. 2. Serum L(+)-tartaric-acid-inhibited acid phosphatase in a variety of diseases in childhood.

and cupric ions (22) and the acid phosphatase of the serum from patients with Gaucher's disease is inhibited mainly by fluoride (5). The last-mentioned phosphatase also splits only disodium phenyl phosphate substrate but not beta-glycerol phosphate. Certain studies make the above classification questionable; for example, tartaric-acid-inhibited acid phosphatase has been found in tissues other than the prostate and in the serum of both human and animal females (9, 17,

18). The present study also bears on these facts.

During the process of growth, infants and children have a higher serum acid-phosphatase activity than adults (18). This was demonstrated by using as substrate disodium phenyl phosphate but was not evident when beta glycerophosphate was used (11).

Only occasional reports have been made of increased serum acid-phosphatase activity in children. These observations con-

cern few cases of osteopetrosis and osteogenesis imperfecta (24, 10). Recently a rise of serum-total acid phosphatase has been observed in Gaucher's disease (5). No mention of acid-phosphatase activity decreasing below normal range is made in any of the reports.

In the present investigation it was noted that the variation of serum-total acid phosphatase in the conditions listed below was greater than that found in normal children, i.e. in acute rheumatic fever, diseases of the lungs, kidneys and bones. Occasional increased activity in kidney and bone disease has been encountered in adults (6, 8, 13). A most interesting finding was the high incidence of increased total and tartaric-acid-inhibited acid phosphatase in children with acute rheumatic fever. Although no explanation for these observations is at hand at the present time, it seems that the phosphatase activity is linked to the disease process. The fact that the high acid-phosphatase activity decreased in a few instances during clinical improvement supports this assumption. Moreover, the decrease of enzyme activity occurred with either salicylate or prednisone therapy.

Although slightly decreased activity was observed in many of the disorders studied, marked decrease in activity of serum acid phosphatase was observed in some cases of growth retardation, nephritis and one patient with rickets.

The source of the serum phosphatases in both normal state and pathologic conditions is not yet clear. It is worth mentioning that acid phosphatase is also present in other body fluids and an increased activity of this enzyme in CSF has been found in certain neurological

disorders, both in adults (4) and in children (19). Pleural and ascitic fluid in children were also found to contain increased activities of acid phosphatases under certain circumstances (20).

As in previous observations (18), no correlation between serum acid and alkaline phosphatase was found, even in those patients who, suffering from rickets, had high activity of the alkaline phosphatase.

The question why only a certain proportion of cases of a particular disease show a deviation from the normal range of activity of serum acid phosphatases remains unanswered until more data are accumulated and better knowledge concerning the metabolic role of these enzymes is available.

### Summary

Serum-total acid phosphatase (TAP) and L(+)-tartaric-acid-inhibited acid-phosphatase (TIAP) activities have been determined in 95 and 55 children respectively, suffering from a variety of diseases. It was found that in a certain number of children with rheumatic fever, diseases of the liver, lungs, kidneys and bones the variations in total-serum acid phosphatase were considerably greater than in normal children. The activity of TIAP was significantly increased in rheumatic fever.

### Acknowledgement

The authors are indebted to Mrs. M. Dudek and to Dr. M. Gefman for their help in different stages of this study. Mr. A. Bratu kindly drew the figures and Prof. A. de Vries gave helpful criticism during the preparation of the manuscript.



## References

1. ABUL-FADL, M. A. M. and KING, E. J.: Properties of acid phosphatases of erythrocytes and of human prostate gland. *Biochem J*, 45: 51, 1949.
2. BODANSKY, A.: Phosphatase studies: determination of serum phosphatase. Factors influencing accuracy of determination. *J Biol Chem*, 101: 93, 1933.
3. CLAYMAN, C. B., SKLAR, M. and KLAYMAN, M. I.: Acid phosphatase elevation in jaundiced patients. *Gastroenterology*, 33: 245, 1957.
4. COLLING, K. G. and ROSSITER, R. J.: Alkaline and acid phosphatase in cerebro-spinal fluid. *Canad J Res (Sect E)*, 28: 56, 1950.
5. CROCKER, A. C. and LANDING, B. H.: Phosphatase studies in Gaucher's disease. *Metabolism*, 9: 341, 1960.
6. DELORY, G. E., SWEETSER, T. H. and WHITE, T. A.: The use of formalin and alcohol in the estimation of prostatic phosphatase. *J Urol*, 66: 724, 1951.
7. ELLIOTT, G. A., SANDLER, A. and RABINOWITZ, D.: Gonadal dysgenesis in three sisters. *J Clin Endocr*, 19: 995, 1959.
8. EUSIGN, D. C.: Serum phosphatase in osteopetrosis. *J Lab Clin Med*, 32: 1541, 1947.
9. FISHMAN, W. H. and LERNER, F.: A method for estimating serum acid phosphatase of prostatic origin. *J Biol Chem*, 200: 89, 1953.
10. GEBALA, A.: Acid hyperphosphatasia in three families with osteogenesis imperfecta. *Lancet* 2: 1084, 1956.
11. GEBALA, A. and ROSENMUND, H.: Studies on the activity of acid phosphatases in serum of children. *Helv Paediat Acta*, 14: 511, 1959.
12. GUTMAN, A. B. and GUTMAN, E. B.: "Acid" phosphatase activity of the serum of normal human subjects. *Proc Soc Exp Biol Med*, 38: 470, 1938.
13. GUTMAN, A. B., GUTMAN, E. B. and ROBINSON, J. N.: Determination of serum "acid" phosphatase activity in differentiating skeletal metastases secondary to prostatic carcinoma from Paget's disease of bone. *Amer J Cancer*, 38: 103, 1940.
14. HASTRUP, B. and VIDEBAEK, A.: Acid phosphatase in Niemann-Pick's disease and a therapeutic experiment with cortisone. *Acta Med Scand*, 149: 287, 1954.
15. KING, E. J. and WOOTON, I. D. P.: Micro-Analysis in Medical Biochemistry, 3rd ed., p. 90. J. & A. Churchill Ltd., London, 1956.
16. KLENDSHOJ, N. C. and KOEFF, G. H.: Serum phosphatase activity in hyperparathyroidism. *J Clin Endocr*, 3: 351, 1943.
17. LARON, Z. and EPSTEIN-HALBERSTADT, B.: Total and L(+)-tartaric-acid-inhibited rat serum acid phosphatase. *Nature* 187: 67, 1960.
18. — Activity of acid phosphatase in the serum of normal infants and children. *Pediatrics*, 26: 281, 1960.
19. LARON, Z. and KOWADLO, A.: Acid phosphatase activity in cerebro-spinal fluid in childhood. To be published.
20. — Unpublished data.
21. LIPMANN KESSEL, A. W. and GORDON SIGNY, A.: Acid hyperphosphatasia in three families with osteogenesis imperfecta. *Lancet* 2: 1217, 1956.
22. REYNOLDS, M. D., LEMON, H. M. and BYRNES, W. W.: Copper resistant acid phosphatase. *Cancer Res*, 16: 943, 1956.
23. TUCHMAN, L. R. and SWICK, M.: High acid phosphatase level indicating Gaucher's disease in patient with prostatism. *JAMA*, 164: 2023, 1957.
24. TURANO, A. F., FAGAN, K. A. and CORBO, P. A.: Variations in chemical manifestations of osteopetrosis. *J Pediatr*, 44: 688, 1954.
25. WALKER, B. S., LEMON, H. M., DAVISON, M. M. and SCHWARTZ, M. K.: Acid phosphatases. *Amer J Clin Path*, 24: 807, 1954.
26. WOODARD, H. Q.: The clinical significance of serum acid phosphatase. *Amer J Med*, 27: 902, 1959.

Submitted Nov. 8, 1960

Accepted Dec. 12, 1960

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## Time Intervals in the Electrocardiogram of Healthy Infants

by ERIC N. COLEMAN

### Material and Methods

One hundred subjects were examined, twenty-five in each quarter of the first year of life. Fifty-eight were boys and forty-two, girls. They were examined during visits to the Child Welfare Clinic at the hospital. None showed clinical evidence of heart disease and in none was there any reason to suspect a disorder of mineral metabolism.

From ten subjects two electrocardiograms were obtained at an interval of 10 to 18 weeks. The measurements used for analysis were taken from the second electrocardiogram but certain comparisons were made between the two records.

The electrocardiogram was recorded with the infant in the recumbent position. No sedative was given. The standard limb leads were recorded simultaneously by means of a photographic electrocardiograph which was standardised so that a current of one millivolt produced beam deflections of one centimetre.

Measurements were made of the length of the cardiac cycle (RR' interval), P wave, PR (PQ) interval, QRS complex, T wave and QT interval and expressed to the nearest 0.01 second. The heart rate was calculated using the length of the cardiac cycle, and if its length varied an average value was taken. The PR interval was measured in standard Lead II. Difficulty was sometimes experienced in separating the T wave from the ST segment. The wave was held to commence where the even course of the ST segment was interrupted by a distinct change in direction or angulation, and this determination was

A substantial difference exists between the electrocardiogram of the child and that of the adult (16). Some investigators have described the variations in the electrocardiogram throughout childhood (5, 12, 23) while others have paid particular attention to its variations during infancy (7, 11, 17). These descriptions make it clear that the greatest amount of variation in the electrocardiogram of the healthy child takes place during the first year of life, and for this reason normal or average standards must be known so that the abnormal may be recognized.

This investigation is concerned only with the length of waves and intervals in infancy. It was prompted by difficulties encountered in the interpretation of changes accompanying electrolyte disturbances, particularly in idiopathic hypercalcaemia of infancy (6). The investigation was confined to the standard limb leads in the belief that these should supply adequate information when evidence of an electrolyte disorder is being sought. Moreover, greater knowledge of normal measurements might be expected to increase the value of the limb leads especially in the outpatient department where praecordial leads may be difficult to record, particularly if a photographic electrocardiogram is used.

facilitated by comparing the synchronously recorded leads. Where the length of the P wave, QRS complex or T wave varied from lead to lead it was measured in that lead in which it was longest. When the length of the QT interval varied an average value was calculated. The measured QT interval was corrected to compensate for the influence of heart rate by the application of Bazett's equation (4), and the term QTc used to designate the resulting value; this calculation was made by slide-rule.

The length of the ST segment was obtained by subtracting the sum of the QRS complex and the T wave, measured in the lead in which they were longest, from the average QT interval. Thus subsequent calculations were based on the shortest ST segment as recommended by Ashman & Hull (3).

The range, arithmetic mean and standard deviation of the mean (S.D.) of the heart rate and intervals were calculated for each 3-month period and for the entire sample. Correlation coefficients ( $r$ ) were calculated for the P wave, PR interval, QRS complex, T wave, QT interval and ST segment against heart rate (beats/minute) and age (weeks). These were tested for significance.

## Results and Discussion

### Heart Rate

The lowest and highest heart rates were 102 per minute at the age of 40 weeks and 207 per minute at the age of 2 weeks. The mean rate for the entire sample was 141 per minute, but in the fourth quarter the

mean rate was 20 beats per minute less than in the first quarter (Table 1). An unexpected finding was that the mean rate in the third quarter was less than in the fourth. Nevertheless a highly significant negative correlation ( $p < 0.01$ ) of heart rate with age was disclosed ( $r = -0.2921$ ).

In 47 subjects the heart rate was between 120 and 139 per minute and in 80 between 100 and 159 per minute. Of the 20 subjects whose heart rate was 160 or over, 11 were less than 3 months old and 6 were between 3 and 6 months old.

It is obvious that these heart rates cannot be regarded as basal or resting values because some subjects were excited by the disturbance inherent in the recording procedure. Nevertheless, as Ziegler (23) has pointed out, such measurements give a useful indication of the wide range through which the heart rate may vary in health. No one would deny that the heart rate should be counted during sleep, but in infancy this is not always practicable. The practice at this hospital is to make the count by auscultation rather than by palpation of a peripheral pulse which is not only less accurate but sometimes impossible. The disadvantage of auscultation is that the baby is often awake and restless by the time the praecordium is exposed.

TABLE 1. Heart rate (beats per minute) and mean values of cardiac cycle (seconds).

Age group	No. of observations	Range of observations	Arithmetic mean	S.D.	Arithmetic mean of cardiac cycle
2 weeks-3 months	25	118-207	155.6	23.32	0.39
3-6 months	25	118-200	143.2	21.47	0.42
6-9 months	25	115-182	130.5	13.52	0.46
9-12 months	25	102-200	135.6	22.88	0.44
2 weeks-12 months	100	102-207	141.2	22.31	0.42

TABLE 2. *P* wave (seconds).

Age group	No. of observations	Range of observations	Arithmetic mean	S.D.
2 weeks-3 months	25	0.04-0.07	0.058	0.0060
3-6 months	25	0.06-0.08	0.066	0.0071
6-9 months	25	0.06-0.08	0.066	0.0057
9-12 months	25	0.06-0.08	0.069	0.0122
2 weeks-12 months	100	0.04-0.08	0.065	0.0081

Published figures for the heart rate of healthy infants show wide differences but a feature common to most is a declining rate with increasing age.

#### *Arrhythmia*

Sinus arrhythmia was the only disturbance of rhythm encountered. The diagnosis, based on the criterion proposed by Krumbhaar & Jenks (14), was made only if the greatest difference between cardiac cycles was 0.1 second or more. Five examples were found. The subjects were 4, 7, 9, 10 and 11 months old, and had average rates of 128, 120, 102, 110 and 133 per minute, respectively. The infrequency of sinus arrhythmia in this sample lends support to the opinion of several authors, including Lincoln & Nicolson (15), that this arrhythmia is less common in infancy than in later childhood and that its incidence rises as the heart rate falls.

The infant with sinus arrhythmia at the age of 10 months had been examined 11 weeks before. In the earlier record the difference of 0.04 second between cardiac cycles was insufficient to permit a diagnosis of sinus arrhythmia. There was little difference between the average heart rates of the two records (115 in the first, 110 in the second), and this suggests that slowing

of the pacemaker is not the sole factor causing sinus arrhythmia.

A difference between cardiac cycles of less than 0.1 second was found in 69 electrocardiograms, but unlike true sinus arrhythmia the incidence was not substantially greater in older infants. There were 17 examples in the first quarter, 16 in the second, 19 in the third and 17 in the fourth.

#### *P* wave

The duration of the *P* wave ranged from 0.04 to 0.08 second, and the mean values increased with age (Table 2). A highly significant positive correlation was found between the length of the wave and age ( $r = +0.5045$ ). This lengthening of the *P* wave with increasing age accords with Ziegler's findings (23), but his mean values are as much as 0.012 second less. The range of values encountered in the present series is precisely the same as that quoted for the whole of childhood by Hafkesbring *et al.* (12). There was no significant correlation between the length of the *P* wave and the heart rate ( $r = -0.1003$ ). It seems probable that the increasing width of the *P* wave observed during the first year of life reflects the increasing mass of the atria.

TABLE 3. *PR interval (seconds).*

Age group	No. of observations	Range of observations	Arithmetic mean	S.D.
2 weeks-3 months	25	0.08-0.14	0.099	0.0127
3-6 months	25	0.08-0.13	0.102	0.0111
6-9 months	25	0.09-0.14	0.111	0.0138
9-12 months	25	0.08-0.13	0.105	0.0172
2 weeks-12 months	100	0.08-0.14	0.104	0.0134

*PR interval*

The average duration of the PR interval for the sample was 0.104 second and the range of individual values was from 0.08 to 0.14 second (Table 3). The mean PR interval lengthened from the first to the third quarter, but was less in the fourth quarter than in the third. This suggested that the PR interval might be more related to heart rate than to age because, as already noted, the mean heart rate was greater in the fourth than in the third quarter. There was a significant correlation ( $p < 0.05$ ) between the length of the PR interval and both heart rate ( $r = -0.2302$ ) and age ( $r = +0.2034$ ), but the influence of the heart rate was the greater, as indicated by the linear regression equation of PR on age and heart rate, viz:—  $PR = 0.000148316 \times \text{Age (weeks)} - 0.000117216 \times \text{Heart Rate (beats per minute)} + 0.117111$ . Thus the PR interval tends to lengthen both with decreasing

heart rate and with increasing age. Alimurung & Massell (2) came to the same conclusion but Savilahti (19) and others have doubted whether the PR interval is influenced by heart rate. The mean values obtained for the length of the PR interval are lower than those given by Burnett & Taylor (5) and by Epstein (8), but agree closely with those of Ziegler (23).

*The ventricular complexes*

The length of the QRS complex in the entire sample varied from 0.05 to 0.08 second, and the mean value was 0.062 second (Table 4). The mean values increased progressively from the first to the fourth quarter. Highly significant correlations ( $p < 0.01$ ) existed between the length of the QRS complex and both age ( $r = +0.5287$ ) and heart rate ( $r = -0.4351$ ). With decreasing heart rate or increasing age the QRS complex lengthens and this finding is in agreement with that

TABLE 4. *QRS complex (seconds).*

Age group	No. of observations	Range of observations	Arithmetic mean	S.D.
2 weeks-3 months	25	0.05-0.07	0.057	0.0069
3-6 months	25	0.05-0.07	0.059	0.0057
6-9 months	25	0.05-0.07	0.064	0.0064
9-12 months	25	0.06-0.08	0.067	0.0062
2 weeks-12 months	100	0.05-0.08	0.062	0.0074

TABLE 5. *T wave (seconds).*

Age group	No. of observations	Range of observations	Arithmetic mean	S.D.
2 weeks-3 months	25	0.09-0.14	0.111	0.0136
3-6 months	25	0.10-0.15	0.115	0.0129
6-9 months	25	0.11-0.15	0.126	0.0112
9-12 months	25	0.11-0.15	0.129	0.0139
2 weeks-12 months	100	0.09-0.15	0.120	0.0146

of Savilahti (8). The mean values given by Ziegler (23) are higher and do not show the same gradual lengthening with age. On the other hand, Seham (21) and Burnett & Taylor (5) reported values which were much lower and Seham's are so low that it seems possible that he may have ignored shallow Q waves when making his measurements. The present figures are however closely similar to those of Jundell & Stenström (13).

The length of the T wave lay between 0.09 and 0.15 second, and the average value for the whole sample was 0.120 second (Table 5). The mean values increased progressively from the first to the fourth quarter. This trend is in agreement with the findings of Ziegler (23) but his mean values are much greater. A highly significant degree of correlation ( $p < 0.01$ ) existed between the duration of the T wave and both age ( $r = +0.3288$ ) and heart rate ( $r = -0.7124$ ) but the

influence of rate was by far the greater. With decreasing heart rate or increasing age the T wave lengthens.

Both the QRS complex and the T wave lengthen throughout the first year of life, and it seems likely that this is a reflection of the growth of the ventricles.

#### *QT interval*

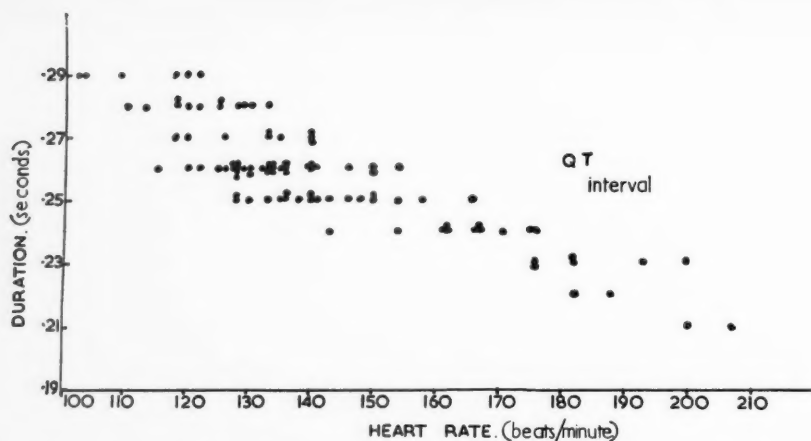
The QT interval or electrical systole includes the QRS complex, the ST segment and the T wave.

The range through which the QT interval varied during infancy was found to be 0.21 to 0.29 second, with a mean value of 0.257 second (Table 6).

Bazett (4) recognised that the length of of the QT interval was affected by the heart rate, and in the belief that the relationship could be expressed by a straight-line graph, he introduced a formula to express such an association. Some investigators have suggested that the rela-

TABLE 6. *QT interval (seconds).*

Age group	No. of observations	Range of observations	Arithmetic mean	S.D.
2 weeks-3 months	25	0.21-0.28	0.250	0.0186
3-6 months	25	0.21-0.28	0.252	0.0141
6-9 months	25	0.22-0.29	0.264	0.0150
9-12 months	25	0.23-0.29	0.264	0.0175
2 weeks-12 months	100	0.21-0.29	0.257	0.0174



Scatter diagram relating 100 measurements of the QT interval to the prevailing heart rate.

tionship is not linear and have devised other methods for relating the measured QT interval to the heart rate (3, 9, 10, 20).

The individual measurements of the QT interval were plotted against the heart rate and it was obvious that the QT interval lengthened as the heart rate fell (Figure). This is shown statistically by a very significant negative correlation ( $r = -0.8572$ ). A significant positive correlation existed between the QT interval and age ( $r = +0.3275$ ) but the influence of age was much less than that of rate as shown by the linear regression of QT on age and heart rate, viz:  $0.000104393 \times \text{Age (weeks)} - 0.000649885 \times \text{Heart Rate (beats per minute)} - 0.347013$ .

The range of QTc values for the whole period under review was 0.36 to 0.42, with an average of 0.392 (Table 7). A survey of the findings of other investigators discloses appreciable differences among their mean values. Epstein (8) accepted a value of 0.38. Alimurung *et al.* (1) and Yu, Joos & Katsampes (22) accepted a value of 0.404. Ziegler (23) found mean values at various ages from one week to one year to lie between 0.385 and 0.408 and the scatter of values was greater than in the present series in which 76 % of all values are within the range, mean  $\pm 1 \times \text{S.D.}$ , and 97 % within the range, mean  $\pm 2 \times \text{S.D.}$

TABLE 7. QTc.

Age group	No. of observations	Range of observations	Arithmetic mean	S.D.
2 weeks-3 months	25	0.370-0.415	0.399	0.0116
3-6 months	25	0.365-0.410	0.388	0.0110
6-9 months	25	0.360-0.415	0.390	0.0153
9-12 months	25	0.375-0.420	0.393	0.0134
2 weeks-12 months	100	0.360-0.420	0.392	0.0133



TABLE 8. *ST segment (seconds).*

Age group	No. of observations	Range of observations	Arithmetic mean	S.D.
2 weeks-3 months	25	0.06-0.11	0.081	0.0153
3-6 months	25	0.06-0.10	0.078	0.0116
6-9 months	25	0.05-0.10	0.075	0.0126
9-12 months	25	0.05-0.08	0.068	0.0082
2 weeks-12 months	100	0.05-0.11	0.075	0.0127

*ST segment*

The length of the ST segment was examined because this portion of the QT interval appears to be particularly susceptible to changes in the concentration of calcium in the body fluids. It seemed possible that measurement of the ST segment might permit the recognition of significant changes in its length too small to lengthen or shorten the entire QT interval to an appreciable extent.

The ST segment was found to vary between 0.05 and 0.11 second with a mean value of 0.075. It was observed from the mean values for the four quarters that this interval shortened with increasing age (Table 8). Highly significant negative correlations ( $p < 0.01$ ) existed between the length of the segment and both age ( $r = -0.3554$ ) and heart rate ( $r = -0.2583$ ). In other words, the ST segment tends to shorten both with increasing age and heart rate.

Thus in this sample both the QRS complex and the T wave lengthened as age increased but the ST segment shortened. This finding was supported from a comparison of the components of the QT interval of the 10 subjects twice examined. In the later records the QRS complex had lengthened or remained unchanged in 10

subjects, the T wave in 7, but the ST segment had shortened or remained unchanged in 8.

## Summary and Conclusions

The standard limb leads of the electrocardiograms of 100 healthy infants were examined. The heart rate and the length of the P wave, PR interval, QRS complex, T wave, ST segment, QT interval and the corrected QT interval (QTc) were measured. The heart rate showed wide variations throughout the period under review but decreased as age advanced. Sinus arrhythmia was encountered only five times but minor differences in the length of cardiac cycles occurred frequently. No other disturbance of rhythm was observed. The P wave, PR interval, QRS complex and T wave lengthened as age increased and, the P wave excepted, as the heart rate slowed. The ST segment tended to shorten both with increasing age and increasing heart rate. The linear association between the length of the QT interval and the heart rate was confirmed. The observed values for the corrected QT interval (QTc) fell within the range, 0.36 to 0.42.

## Acknowledgements

I wish to express my indebtedness to Professor Stanley Graham for his advice

and criticism during the preparation of this paper, to Dr. R. A. Robb, Mitchell Lecturer on the Methods of Statistics for invaluable assistance with the analysis of data, to Dr. Margaret D. Giles for so readily making available the material for the investigation,

to the staff of the Department of Photography at the Royal Hospital for Sick Children for the developing of electrocardiograms and not least to the mothers for their co-operation and the infants for their participation.

### References

1. ALIMURUNG, M. M., JOSEPH, L. G., CRAIGE, E. and MASSELL, B. F.: The Q-T interval in normal infants and children. *Circulation*, 1: 1329, 1950.
2. ALIMURUNG, M. M. and MASSELL, B. F.: The normal P-R interval in infants and children. *Circulation*, 13: 257, 1956.
3. ASHMAN, R. and HULL, E.: Essentials of Electrocardiography. 2nd ed. The Mac-Millan Co., New York. p. 139, 162, 1945.
4. BAZETT, H. C.: An analysis of the time-relations of electrocardiograms. *Heart* 7, 353, 1920.
5. BURNETT, C. T. and TAYLOR, E. L.: Electrocardiograms on 167 average healthy infants and children. *Amer Heart J*, 11: 185, 1956.
6. COLEMAN, E. N.: Electrocardiographic changes in idiopathic hypercalcaemia of infancy. *Brit Med J*, 2: 467, 1959.
7. ENGEL, E.: Das Elektrokardiogramm des gesunden Frühgeborenen, Neugeborenen und Säuglings. *Z Kinderhk*, 59: 359, 1937-38.
8. EPSTEIN, N.: The heart in normal infants and children. *J Pediat*, 32: 39, 1948.
9. FRIDERICIA, L. S.: Die Systolendauer im Elektrokardiogramm bei normalen Menschen und bei Herzkranken. *Acta Med Scand*, 53: 469, 489, 1920.
10. FUNARO, N. and NICOLAI, G.: Das Elektrokardiogramm des Säuglings. *Zbl Physiol*, 22: 58, 1908.
11. GROEDEL, F. M. and MILLER, M.: Electrocardiographic studies in the newborn. *Exp Med Surg*, 2: 110, 1944.
12. HAFKESBRING, ELEANOR M., DRAWE, CATHERINE E. and ASHMAN, R.: Children's electrocardiograms. I. Measurements for 100 normal children. *Amer J Dis Child*, 53: 1457, 1937.
13. JUNEDELL, I. and STENSTRÖM, N.: A study of the electrocardiogram in infants of normal conditions and during treatment with cod liver oil and vigantol. *Acta Paediat*, 12: 113, 1931.
14. KRUMBHAAR, E. B. and JENKS, H. H.: Electrocardiographic studies in normal infants and children. *Heart*, 6: 189, 1915.
15. LINCOLN, E. M. and NICOLSON, G. H. B.: The hearts of normal children. III. Electrocardiographic records. *Amer J Dis Child*, 35: 1001, 1928.
16. MANNHEIMER, E.: Calibrated Phonocardiography and Electrocardiography. *Acta Paediat*, 28: Suppl. 44, 1940.
17. NÁDRAI, A.: Die Elektrokardiographie im Säuglingsalter. *Z Kinderhk*, 60: 285 1938.
18. SAVILAHTI, M.: Concerning the effect of frequency and age in the QRS time of the electrocardiogram. *Acta Med Scand*, 123: 143, 1946.
19. SAVILAHTI, M.: On the normal and the pathological PQ time of the electrocardiogram. *Acta Med Scand*, 123: 252, 1946.
20. SCHLAMOWITZ, I.: An analysis of the time relationships within the cardiac cycle in electrocardiograms of normal men. I. The duration of the QT interval and its relationship to the cycle length (R-R interval). *Amer Heart J*, 31: 329, 1946.
21. SEHAM, M.: Electrocardiography in children. *Abt's Pediatrics* Vol. IV. W. B. Saunders Co., Philadelphia, p. 282, 1924.
22. YU, P. N. G., JOOS, H. A. and KATSAMPES, C. P.: Unipolar electrocardiogram in normal infants and children. *Amer Heart J*, 41: 91, 1951.
23. ZIEGLER, R. F.: Electrocardiographic Studies in Normal Infants and Children. Charles C. Thomas. Springfield, Ill. 1951.

Submitted Oct. 26, 1960

Accepted Nov. 15, 1960.

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## Fractional Collection of Urine in Very Small or Ill Infants

by R. L. CORT

Many methods of collecting urine in infants have been described. Each has certain advantages, the most important of which is probably the familiarity of the nursing and medical staff with a given technique.

The method to be described was worked out to fulfil certain requirements: simplicity, so that it can be carried out by nurses with other routine ward duties, measurement of very small quantities of urine at relatively short intervals, and absence of danger of skin or other trauma, particularly in critically ill infants.

Its chief use has been in healthy and ill newborn premature infants, 0-5 days of age, although full-term infants 0-24 hours of age have also been studied. No provision for the collection of stools (meconium) is made.

### Method

For collection of urine samples, the equipment shown in Fig. 1 is used. It consists of:

1. A pad of washed absorbent cotton. The cotton is weighed, washed with 0.1 N sulphuric acid, then with distilled water, and is then suction- and heat-dried. A weight of 3 g was chosen for convenience. This amount of cotton will absorb about 30 ml of urine without leakage.

2. A thin rubber cap into which the cotton fits

3. A pair of simple holding panties with four tie fastenings, which permit examination of the cotton, cleaning, rectal temperature measurement, etc., with minimal disturbance of the infant.

4. A numbered glass weighing bottle containing 20-25 ml of water or other eluting fluid.

The washed cotton is fitted into the rubber cap and placed on the infant's perineum (Fig. 2). With either sex the cotton is hollowed out to admit the penis or the labia. If avoidance of contamination with meconium is absolutely necessary, a small strip of non-irritant adhesive tape may be used to fix the posterior margin of the cap to the perineal body. In practice, contamination with stools is uncommon or very slight.

A folded diaper is placed over the cap and through the infant's legs, and held in place by the panties. Thus, slight pressure is exerted to hold the cotton in place. Newborn premature infants, particularly ill ones, move very little, and our newborn infants are swaddled, so that displacement of the cotton wad through the infant's activity is rare.

Every three hours, at feeding time, the nurse unties the panties and lifts the upper edge of the cap to see if urine has been passed. If the cotton pad is wet, she removes it and places it in a weighing bottle, the number of which is noted. The infant may be weighed with the collecting cotton in place.

For estimating the urinary constituents, the bottle is weighed to determine the volume of urine, the cotton thoroughly

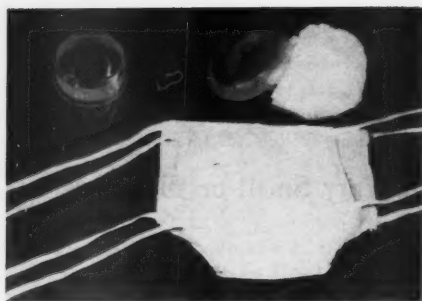


Fig. 1



Fig. 2

squeezed out in the eluting fluid, and determinations are carried out on the diluted urine.

### Comment

This method of urine collection has limited but real usefulness. It is surprisingly accurate, and has proved of value in very small or ill infants, where cumbersome or possibly traumatic urine collection would be out of the question. It also does not require constant attention by research nurses.

Submitted Nov. 28, 1960

Accepted Dec. 16, 1960

Recovery of volume is about  $\pm 0.3$  ml. Recovery of urinary constituents is satisfactory, although calculation of urinary concentrations in volumes below 1 ml is inaccurate. Blanks are small for most common urinary constituents, and may be decreased by modifying the washing procedure or using other absorbent materials in place of cotton. This question has not been exhaustively examined, however.

Routine care is simple and rapid, and metabolic balances have not proved burdensome, even on a busy premature ward.

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## Clinodactyly and Brachymesopthalgia of the Fifth Finger

by A. F. ROCHE

### Review of Literature

The term clinodactyly is applied to a curvature of the extended finger and, in the case of the fifth finger, this curvature is always towards the fourth. A slight curving in this direction is normal and earlier workers have restricted the use of this term to cases of marked inclination, determined on a basis of subjective judgement. By brachymesopthalgia is meant a shortness of the shaft of the middle phalanx. Schmid & Junker (10) have suggested a definition of brachymesopthalgia based on measurements of phalangeal shaft length. Other workers have subjectively assessed the length of the middle phalangeal shaft relative to the lengths of the other phalangeal shafts in the digit and the size of the hand and have used their judgement to determine the amount of shortness necessary for the application of this term. During this investigation similar subjective methods have been followed before the term brachymesopthalgia has been used.

Many have accepted without question the statement by Smith (11) that marked clinodactyly of the fifth finger, associated with a shortness of its middle phalanx, is present in nearly every mongoloid. The literature, however, contains few investigations of this alleged relationship in either mongoloid or normal children.

Reports of the incidence of clinodactyly in mongoloids vary between 34.6% and 78.8% (Table 1) after the omission of the small series examined by West (15). The incidence of clinodactyly in normal children has been reported as 19.5% (15) and 5.2% (2) (Table 1). It is probable that much of this variation is due to the lack of a precise determination of normal limits and an application of terms based upon subjective criteria.

Clinodactyly could occur at either or at both the interphalangeal joints of the digit. There is no information in the literature concerning the common site of clinodactyly except the observation of Thursfield (13) that clinodactyly of the fifth finger in mongoloids occurred at the distal interphalangeal joint.

Brachymesopthalgia of the fifth finger has been reported in 62 of 100 mongoloids (3) and in 10 of 700 normal individuals (12). Hefke (3) reported clinodactyly in 43 of 62 mongoloids in whom brachymesopthalgia was present in the same digit. Because this is a higher incidence of clinodactyly than in the general group of mongoloids he studied, he concluded that these conditions are often associated. Opinions that clinodactyly and brachymesopthalgia tend to occur in combination have also been expressed by Pol (8) for mongoloids and by West (15) for normal children.

It has been stated that clinodactyly increases with age in mongoloids (1, 15) although this is denied by Øster (7). Hefke (3) claimed that brachymesopthalgia became less marked in mongoloids with advance in age.

In the present study an attempt has been made to evaluate the possible association between clinodactyly and brachymesophaia in the fifth fingers of both mongoloid and normal children.

### Material and Methods

The selection of 192 mongoloid and 120 normal children has been described elsewhere (9). Observations have been made on serial dorso-palmar radiographs of the left hand of these children taken at regular intervals over a four year period. Only those radiographs have been utilized in which the fingers were extended fully and abducted slightly from each other. Clinodactyly and brachymesophaia of the fifth finger have been identified in a mixed sequence of mongoloid and normal children by the same observer using subjective criteria similar to those of other workers.

### Findings and Discussion

At all ages studied, clinodactyly was more common in mongoloid than in normal children (Table 2). Combining both sexes and all ages, the incidence was

55.2% for mongoloids and 32.5% for normal children. These findings are similar to those reported previously for mongoloids, but are higher than those reported previously for normal children (Table 1). Such observations do not justify the claim of Smith (11) that "this curve of the little finger occurs to a greater or less degree in nearly every case of mongol idiocy".

Clinodactyly occurred most commonly at the distal interphalangeal joint in mongoloids, which is in agreement with the observation of Thursfield (13). In normal children, however, it was slightly more common for both the proximal and distal interphalangeal joints to be involved (Table 2). This difference between the mongoloid and the normal children in the site of clinodactyly occurred even in those in whom the fifth middle phalanx was of the usual length and, consequently, it could not have been dependent upon the marked difference between the mongoloid and normal children in the incidence of brachymesophaia. In both mongoloid

TABLE 1. *The recorded incidence of clinodactyly.*

Author	Number examined	Number with clinodactyly	% with clinodactyly
<i>Mongoloid children</i>			
West (1901)	9	1	11.1
Muir (1903)	26	9	34.6
Thursfield (1921)	26	13	50.0
Orel (1927)	26	33	78.8
Hefke (1940)	100	43	43.0
Benda (1949)	*	*	36.2
Engler (1949)	*	*	40.0
Oster (1953)	504	252	50.0
Levinson <i>et al.</i> (1955)	50	34	68.0
<i>Normal children</i>			
West (1901)	605	118	19.5
Engler (1949)	500	26	5.2

\* Not recorded by author.

TABLE 2. *Males/Females.*

Chronological age in years	Total children	Site of clinodactyly			Percentage with clinodactyly	No. with brachymesophalangia
		Proximal interphalangeal joint only	Distal interphalangeal joint only	Both interphalangeal joints		
<i>Mongoloid children</i>						
0-2	7/9	0/0	4/4	0/0	57.1/44.4	3/5
2-7	46/46	2/2	14/19	5/1	45.7/47.8	10/9
8+	37/47	4/4	12/28	6/1	59.5/70.2	11/14
Total	90/102	6/6	30/51	11/2	52.2/57.8	24/28
<i>Normal children</i>						
2-7	60/60	1/2	7/9	14/6	36.7/28.3	2/2

and normal children, clinodactyly at both the proximal and distal interphalangeal joints of an individual was more common in males than in females.

Brachymesophalangia was much more common in the mongoloid (27.1 %) than in the normal children (3.3 %) (Table 2). This is lower than the incidence reported for mongoloids by Hefke (3) but is similar to that reported for normal children by Stettner (12). No case of absence of the fifth middle phalanx was observed among either the mongoloid or the normal individuals.

When mongoloid and normal children of similar age were compared (Table 3) it was found that clinodactyly occurred

more commonly in the mongoloid (47.2 %) than in the normal children (32.5 %). However, clinodactyly associated with a middle phalanx of normal length was observed with almost equal frequency in both mongoloids (25.0 %) and normal children (29.2 %). In all children in whom brachymesophalangia was noted clinodactyly was present also with the exception of three mongoloids. The fact that these conditions sometimes occur independently is contrary to the report of Smith (11). Brachymesophalangia associated with clinodactyly was present in 22.2 % of the mongoloid children under the age of 8 years but in only 3.3 % of normal children of comparable age.

TABLE 3. *Findings in children aged less than 8 years.*

Conditions	Mongoloid		Normal	
	No.	%	No.	%
Clinodactyly without brachymesophalangia	27	25.0	35	29.2
Clinodactyly with brachymesophalangia	24	22.2	4	3.3
Brachymesophalangia without clinodactyly	3	2.8	—	—
Neither clinodactyly nor brachymesophalangia	54	50.0	81	67.5
Totals	108	100.0	120	100.0
Total clinodactyly	51	47.2	39	32.5
Total brachymesophalangia	27	25.0	4	3.3



The serial records of both mongoloid and normal children show a remarkable stability for each individual in both the site and the degree of clinodactyly and the extent of brachymesopha-langia. However, brachymesopha-langia became slightly less common as older groups of mongoloids were considered. These findings are in agreement with those of Øster (7) but are contrary to those of West (15) and Brousseau (1) concerning clinodactyly and those of Hefke (3) in regard to brachymesopha-langia.

### Conclusion

A consideration of the above findings leads one to conclude that there are two types of clinodactyly of the fifth finger. This is best illustrated by reference to the findings on mongoloid and normal children of comparable age (Table 3). In some children with clinodactyly the fifth middle phalanx is of normal length. This type of clinodactyly occurs with approximately equal frequency in both mongoloid and normal children, although the common site of such clinodactyly is different in the two groups. In other children, clinodactyly of the fifth finger is associated with brachymesopha-langia and this type is much more common in the mongoloid group (22.2%) than in the normal group (3.3%).

There is a similar marked difference between the mongoloid (25.0%) and the normal groups (3.3%) in the incidence of brachymesopha-langia. The brachymesopha-langia was combined with clinodactyly in all but three of these children. It is suggested that brachymesopha-langia should be used in the diagnosis of suspected

mongolism and that, if this is done, the recording of clinodactyly provides no additional information that is diagnostically useful.

### Summary

1. Serial radiographs of the hands of 192 mongoloid and 120 normal children extending over four years have been used to investigate the frequency of clinodactyly and brachymesopha-langia in the fifth finger and their changes during growth.

2. The incidence of clinodactyly in the mongoloid children was similar to that recorded in the literature but the incidence in the normal children was higher than that recorded previously. Clinodactyly with a middle phalanx of the usual length occurred with almost equal frequency in both mongoloid and normal children. There was a marked difference between the two groups of children in the incidence of clinodactyly associated with brachymesopha-langia.

3. It is suggested that brachymesopha-langia is a more important physical sign of mongolism than clinodactyly. Considering children under the age of 8 years, it was present in 25.0% of the mongoloid group and 3.3% of the normal group and was almost always associated with clinodactyly.

4. The incidence of clinodactyly did not vary with age but brachymesopha-langia was slightly less common among older mongoloids.

### Acknowledgements

This work was supported financially by the Mental Health Research Fund and was assisted by the co-operation of the officers and staff of the Mental Hygiene Authority of Victoria. I am deeply grateful to Professor L. J. Ray for his encouragement and advice.

## References

1. BROUSSEAU, K.: Mongolism. The Williams and Wilkins Co., Baltimore, 1928.
2. ENGLER, M.: Mongolism. John Wright and Sons Ltd., Bristol, 1949.
3. HEFKE, H. W.: Roentgenologic study of anomalies of the hand in one hundred cases of mongolism. *Amer J Dis Child*, 60: 1319-1323, 1940.
4. LEVINSON, A., FRIEDMAN, A. and STAMPS, F.: Variability of mongolism. *Pediatrics*, 16: 43-54, 1955.
5. MUIR, J.: An analysis of twenty-six cases of mongolism. *Arch Pediat*, 20: 161-169, 1903.
6. OREL, H.: Zur Klinik der mongoloiden Idiotie. *Z Kinderheilk*, 44: 449-472, 1927.
7. ØSTER, J.: Mongolism. Danish Science Press Ltd., Copenhagen, 1953.
8. POL, —: "Brachydaktylie"—"Klinodaktylie"—Hyperphalangia und ihre Grundlagen. *Virchow Arch Path Anat*, 229: 388-530, 1921.
9. ROCHE, A. F. and SUNDERLAND, S.: Melbourne University child growth study. *Med J Aust*, 1: April 25, 559-562, 1959.
10. SCHMID, F. and JUNKER, F.: Die Brachymesophalangie des Kleinfingers. *Z Kinderheilk*, 68: 399-407, 1950.
11. SMITH, T. T. A peculiarity in the shape of the hand in idiots of the "mongol" type. *Pediatrics*, 2: 315-320, 1896.
12. STETTNER, E.: Über die Beziehungen der Ossifikation des Handskeletts zu Alter und Längenwachstum bei gesunden und kranken Kindern von der Geburt bis zur Pubertät. *Arch Kinderheilk*, 68: 439-466, 1921.
13. THURSFIELD, H.: Notes on mongolism. *Brit J Child Dis*, 18: 18-21, 1921.
14. WARNER, E. N.: A survey of mongolism with a review of one hundred cases. *Canad Med Ass J*, 33: 495-500, 1935.
15. WEST, J. P. A note on the little finger of the mongolian idiot and of normal children. *Arch Pediat*, 18: 918-924, 1901.

Submitted Sept. 22, 1960

Accepted Oct. 1, 1960

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## Discussion on the Advisability of Performing Exchange Transfusion<sup>1</sup> in Neonatal Jaundice of Unknown Aetiology<sup>1</sup>

by DYRE TROLLE

The indications for exchange transfusion in neonatal jaundice of unknown aetiology have been much discussed in recent years. These procedures are performed because it is known that some of the children will develop cerebral palsy as a consequence of the toxic effect of hyperbilirubinaemia on the nerve cells of the basal ganglia of the brain. Both the anatomical lesions and the clinical signs are the same as those seen in untreated newborn children with haemolytic disease due to Rhesus incompatibility. It is evident from the investigation of Plum (8) of 478 children more than one year of age, suffering from cerebral palsy, that there is an aetiological relationship between severe jaundice (with or without blood-group incompatibility) occurring in the first weeks of life and the subsequent development of athetosis. Other forms of cerebral palsy are probably not due to neonatal jaundice.

The exact bilirubin level at which exchange transfusion should be performed in neonatal jaundice of unknown aetiology is still unsettled. Most authors who recom-

mend this procedure believe that it should be performed if the serum bilirubin level reaches or exceeds 20 mg %. Some estimate that the critical bilirubin level is 25 mg %, others that it is 15 mg %. The lower level, is especially applicable to premature children. The literature on this question has recently been reviewed (4).

### Material and Methods

During the six-month period, April 1 to October 1, 1960, we systematically investigated serum bilirubin values in practically all jaundiced newborns. We examined only the jaundiced children since it is unlikely that newborn infants without jaundice will have a bilirubin value of 10 mg % or more, and it was this group in whom we were interested. Daily serum bilirubin determinations were performed on heel blood. The micromethod used (Jendrassik & Grof) gives the total bilirubin concentration. The analyses were done at the Central Laboratory of Rigshospitalet (Head: Poul Astrup, M.D.).

In addition, serological examinations were carried out on all jaundiced children and their mothers at the Blood Bank of Rigshospitalet (Head: Erik Freiesleben, M.D.).

(a) *The newborn child.* ABO and Rh (D) group, direct Coombs test, and a Munk-Andersen conglutination test if the infant's red cells were found to be incompatible with

<sup>1</sup> This investigation was in part supported by a PHS research grant B-2408 from the National Institute of Neurological Diseases and Blindness, U.S. Public Health Service.

the serum of the mother with respect to the ABO system.

(b) *Compatibility test between the child's red cells and the mother's serum.* The test was performed with a saline suspension in a test tube at 37°C and followed by an indirect Coombs test.

(c) *The mother.* ABO and Rh (D) group. Furthermore, the serum was examined at 37°C for irregular antibodies using saline suspensions from a large panel of known red blood cells, supplemented with an indirect Coombs' test. If ABO group incompatibility was found, the mother's serum was examined for specific haemolysins and immune antibodies against the child's ABO antigen (neutralization technique with indirect Coombs test).

In the six-month period of the study there were 1073 single births. As a prophylactic measure all the children were given 2.5 mg Menadione intramuscularly immediately after birth. In order to calculate the incidence of jaundice, all stillbirths and neonatal deaths were excluded from the total, because all jaundiced children survived the first week of life. This gives us a corrected figure of 1000 newborns.

Of these 1000 newborns, 363 developed jaundice within the first week of life:

- 36, i.e. 3.6%, due to haemolytic disease (morbus haemolyticus ex rhesus incompatibilitate);
- 22, i.e. 2.2%, due to haemolytic disease (morbus haemolyticus ex ABO incompatibilitate);
- 305, i.e. 30%, due to jaundice of unknown aetiology (icterus ex causa ignota).

### Results

Ninety-five of the 305 infants with jaundice of unknown aetiology had serum bilirubin value  $\geq 15$  mg %, and 65 children had a serum bilirubin level of  $\geq 20$  mg %; all had values of 10 mg % or more.

The percentage of primiparae is the same in the group of infants with jaundice

of unknown aetiology as it is in the group without jaundice.

The frequency of prematurity ( $\leq 2500$  g) for the 305 children with jaundice of unknown aetiology was 24.2%, while the frequency for the remaining 695 was 6.5%. (When the 58 who were icteric due to Rhesus or ABO incompatibility are subtracted from the 695, the prematurity frequency is 6.4%.) Frequency of prematurity in the 65 infants with a serum bilirubin value  $\geq 20$  mg % was 20%. Exchange transfusion was carried out in 36 of the 65 children, i.e. 55%; for these the prematurity frequency was 19%.

Thus, in the period under investigation 3.6% of the 1000 newborns had exchange transfusions because of jaundice of unknown aetiology. The ratio was 5.9% of the premature and 3.3% of the full-term infants. All who were not given exchange transfusion had a serum bilirubin concentration less than 25 mg %.

Despite the fact that this case material of our department in many respects is a selected one (e.g., the high number of premature births), the percentage of children with jaundice of unknown aetiology in both the full-term and premature groups is, by our present knowledge, representative of a non-selected group. The very high percentage of exchange transfusions performed in this series leads one to question whether so many are necessary.

It should be noted that the department is a centre for Rhesus immunized mothers, and of the 1000 children during the six-month period plus 1970 corresponding infants from 1959, 3.6% had haemolytic disease due to Rhesus incompatibility. Eighty percent of these children had exchange transfusions, i.e. 2.9% of the

TABLE 1. *Schematic presentation of the calculations. (To be read from the left to the right.)*

Number		
Out of	1,000,000	children aged more than one year
there are	0.2 % = 2,000	with cerebral palsy
out of these 2000	17.0 % = 340	have athetosis
out of these 340	70.0 % = 238	had severe jaundice in the first week of life
out of these 238	46.0 % = 109	had jaundice due to Rhesus-incompatibility
out of these 238	20.0 % = 48	had jaundice due to ABO-incompatibility
out of these 238	34.0 % = 81	had jaundice of unknown aetiology
out of these 81	33.3 % = 27	were mature (that is 0.0028 % of 955,000)
out of these 81	66.7 % = 54	were premature (that is 0.1200 % of 45,000)

2970 children. Even if one takes into account the fact that the department is a Rhesus centre, it will be seen that, in the six-month study period, more infants were given exchange transfusions because of jaundice of unknown aetiology than because of Rhesus incompatibility (3.6 % against 2.9 %).

#### Calculations

In order to answer the question of how necessary it is to perform exchange transfusions in so many children with jaundice of unknown aetiology, the following calculations have been made for 1,000,000 non-selected and non-exchanged live-born infants on the basis of the following information.

According to Hansen's follow-up study (3) the frequency of cerebral palsy in this country is about 0.2 %. In Plum's investigation (8) athetosis is found in 17 % of the children with cerebral palsy. Moreover, in his retrospective study Plum showed that just less than 70 % of the children with athetosis had severe jaundice during the first weeks of life. In 46 % this was due to Rhesus incompatibility, in 20 % to ABO incompatibility, while in 34 % there was no serological

explanation. The incidence of prematurity ( $\leq 2500$  g) was 10 %, 55 % and 66.7 %, respectively.

The incidence of premature births in 1956 in this country was 5.7 % (5). Norregaard's study (7) showed that between 20 and 25 % of the premature births (by his definition:  $< 2500$  g) die in the first year of life. This means that at the age of one year the frequency of premature births is 4.5 %.

Of 1,000,000 newborn 943,000 are full-term and 57,000 premature (5.7 %), but of 1,000,000 children aged one year 955,000 will have been full-term and 45,000 premature (4.5 %).

Do these figures agree with the exchange transfusions actually performed?

#### Rhesus group

First the question must be answered for the Rhesus group. It is well known that maternal Rhesus antibodies are found in 0.5 % of all pregnancies. In a study of Rhesus immunised mothers from this department it was found that 8 % had macerated or hydroptic babies (6). In other words, among 1,000,000 live births there will be 4600 infants of Rhesus immunised mothers. What will happen o

these children if nothing is done for them? Vaughan, Allen & Diamond, (9) state that 34 % will die and 5 % of the survivors will have kernicterus. Of the above-mentioned 4600 children, 1565 will die, 151 will survive but will have kernicterus, and 2885 will be normal.

Since kernicterus manifests itself most frequently as athetosis, there is good correlation between the number of children with kernicterus, which we find within the untreated Rhesus group, and the number of children with athetosis we estimate on the basis of Plum's retrospective study (151 and 109, respectively).

How many of these 4600 children would have been exchange transfused in our department? According to the above mentioned Rhesus material (6), this number would be 63.0 % since 12.9 % would be Rhesus negative, 4.5 % Rhesus positive but not sensitised, and 19.6 % Rhesus positive and sensitised but exchange transfusion would be considered unnecessary. In the same study we found that three of the 249 exchange transfused children died for unknown reasons during or immediately after an uncomplicated exchange transfusion, in spite of the fact that these children were no more sick than the remaining 246. We, therefore, must reckon with a mortality rate of 1 % due to the exchange transfusion itself.<sup>1</sup> In

other words, 2898 infants would have been exchanged in order to avoid kernicterus in 151 and death in 1564, but the exchange transfusion itself might have caused the death of 29 of these. Although we exchange transfuse slightly more than necessary, there is no doubt that our indications for exchange transfusion are appropriate and sound.

#### *Children with jaundice of unknown aetiology*

It can be seen from the calculations in Table 1 that athetosis in children suffering from jaundice of unknown aetiology in the first weeks of life is 43 times more frequent in premature babies than in full-term babies (0.12 % vs. 0.0028). Therefore the question of the necessity of exchange transfusion has to be investigated separately for the full-term babies (>2500 g) and the premature babies ( $\leq$ 2500 g).

##### *(a) Full-term babies*

In the period under investigation 881 of the 1000 children were full-term. Jaundice of unknown aetiology was found in 231 or 26.0 %. Serum bilirubin values of  $\geq$  20 mg % were found in 52 (5.9 %) of the 881 children, and 29 of the 52 children (56 %) were exchange transfused, i.e. 3.3 % of the 881.

Applying these figures to the hypothetical 943,000 newborn full-term babies will mean that 247,066 will have a serum bilirubin of  $\geq$  10 mg % during the first

particularly important in infants with hyperbilirubinemia not primarily the result of hemolytic disease, such as hyperbilirubinemia in prematures, for which the role of exchange transfusions has been less well evaluated than it has in cases of erythroblastosis fetalis." (Wolff, James A.: Discussion: *Bull Sloane Hosp Women*, 5: 95-97, 1959.)

<sup>1</sup> Similar unexplainable deaths among Rh-sensitised children who had exchange transfusions have been reported from the Sloane Hospital. "From 1947 through 1955, of 236 exchange transfusions, there were 4 unexpected deaths, a total of 1.7 per cent. From 1956 through 1958, among 126 such procedures there were 4 unexpected fatalities, a total of 3.2 per cent. Because of these unexplainable deaths it is concluded that one always also should consider the risk of the exchange transfusion itself. "Comparison of the risk involved, however, is



week of life, 55,637 a serum bilirubin of  $\geq 20$  mg %, and 31,119 will be exchange transfused for fear that they will later develop athetosis.

According to the calculations (Table 1) only 0.0028 % will later develop athetosis. To avoid this in 26 cases we would exchange transfuse, 31,119, and if we had adhered strictly to the value of  $\geq 20$  mg %, the number would have risen to 55,637. Since the department had not previously carried out exchange transfusions in children with jaundice of unknown aetiology, and since we know that these children did not die, the enormous discrepancy shows that our indications for exchange transfusions are unsound.

It may, of course, be said that it is better to exchange transfuse too many rather than too few, but here one must take into account the mortality rate in exchange transfusion, which for the full-term babies is calculated to be 1 %. This would mean that if we decided to exchange transfuse all full-term babies with serum bilirubin levels of  $\geq 20$  mg % of unknown aetiology, we would have to exchange transfuse 2140 children in order to prevent one from developing athetosis and would run the risk of losing 21 by the procedure.

#### (b) *Premature babies*

Of the 1000 children in the period under investigation 119 were premature. Jaundice of unknown aetiology was found in 74 (62 %) and serum bilirubin values of  $\geq 20$  mg % in 13, i.e., 11 %<sup>1</sup> of the 119

children, and 7 of the 13 children (54 %) were transfused, i.e. 5.9 % of the 119.

Applying these figures to 57,000 newborn prematures would mean that 35,340 would have a serum bilirubin of  $\geq 10$  mg % in the first week of life, 6270 a serum bilirubin of  $\geq 20$  mg %, and 3363 would be exchange transfused to avoid the possible development of athetosis.

According to the calculations (Table 1), 0.12 % should later develop athetosis, i.e. 68 of the 57,000 premature infants. Thus, to avoid athetosis in 68, 3363 would be exchange transfused, or 6270 if the limit of  $\geq 20$  mg % is strictly adhered to. We would thus have to exchange transfuse 92 children in order to save one. From the Rhesus material in this department (6) we know that exchange transfusion carries a mortality rate of 4 % in premature babies. In other words, if we decide to exchange transfuse all prematures with a serum bilirubin of  $\geq 20$  mg % of unknown aetiology, we run the risk of having 4 infants die for each one we prevent from developing athetosis by performing an exchange transfusion.

The conditions are, however, difficult to evaluate because it is quite possible that there are more children with athetosis among those dying in the first year of life than among the survivors. But how many? From the Rhesus group we know that if we choose not to exchange transfuse it means that a number corresponding to a good 10 times the number with kernicterus will die. If we use this number, we would save  $68 + 704 = 772$  by exchange transfusing 6270, i.e., save 10 for each 90 transfused, but we would run the risk of 4 dying because of this procedure.

<sup>1</sup> This figure corresponds exactly to that found by Dyggve (1) after prophylactic treatment of premature children with 1 mg  $K_1$  vitamin given intramuscularly immediately after birth.



### ABO group

Finally, the question has been investigated with reference to the ABO group. In our material of 1000 children there were 22 jaundiced children in whom A or B antibody could be demonstrated (2.2 %). (This figure agrees with an earlier consecutive study of 3,500 neonates from this department (2) in which 2.0 % with A or B antibody were found, 84 % and 16 %, respectively. As far as the demonstration of A or B antibodies is concerned, both study groups were non-selected.) Of the 22 children 9 were exchanged, i.e. 0.9 % of the 1000 children. Here there is also a great discrepancy between the number of exchange transfusions performed and the figures based on Plum's study. The same considerations mentioned in the section on infants with jaundice of unknown aetiology apply here.

### Discussion

The criteria for exchange transfusion of Rhesus-sensitised babies are well-founded.

Even if one assumes it to be a fact that full-term infants with jaundice of unknown aetiology may later develop athetosis, we do not know today which children should be exchanged transfused. It seems justifiable not to exchange transfuse the children simply because the serum bilirubin values reach 20–25 mg %. If it rises to over 30, 35 or 40 mg % it is, with our present knowledge, questionable whether one dares to omit an exchange transfusion. Both the non-exchange transfused infants and those transfused should be followed up.

Our criteria for performing transfusions on premature infants with jaundice

of unknown aetiology and children with jaundice due to ABO incompatibility are unsatisfactory.

Cases of kernicterus in premature infants with serum bilirubin values as low as 12.6 mg % have been reported (4). Therefore, it does not appear that the serum bilirubin concentration alone is the decisive factor. Other factors must also be involved. For example, it is not yet known whether, it is a rapid rise in serum bilirubin value (e.g. more than 20 mg % within the first 48 hours), the duration of a severe jaundice or a prolonged jaundice alone, that is decisive for the brain damage. Perhaps the amount of indirect bilirubin plays a part. It is possible that the percentage of haemoglobin, reticulocyte count and amount of foetal haemoglobin in the newborn are of importance, and possibly the amount of blood transferred from the placenta to the infant before the cord is tied also plays a part. It is difficult to say, at the moment, what one should do, but the premature infant runs a greater risk of developing athetosis than does the full-term infant. A systematic investigation, including follow-up, must be carried out in order to solve the problem.

### Summary

In a study of 1000 newborns jaundice of unknown aetiology was a common finding during the first week of life. In these children the bilirubin values were as follows:

$\geq 10$ mg % in	$\left\{ \begin{array}{l} 26 \% \text{ of full-term} \\ 62 \% \text{ of premature} \end{array} \right.$
$\geq 20$ mg % in	$\left\{ \begin{array}{l} 6 \% \text{ of full-term} \\ 11 \% \text{ of premature} \end{array} \right.$

It is known from the literature that there is a causal relationship between

severe jaundice occurring in the first week of life and the subsequent development of athetosis. By carrying out exchange transfusion on children with a serum bilirubin value of  $\geq 20$  mg %, and comparing the number with the frequency of athetosis reported in the literature, it is shown that the following will be exchange transfused:

- (a) 2140 full-term babies in order to prevent subsequent athetosis in one, but one runs the risk of 21 dying as a result of the procedure itself.
- (b) 92 premature infants to prevent subsequent athetosis in one, but one runs the

risk of 4 dying as a result of the procedure.

The limit of 20 mg % is thus not appropriate. Through systematic investigations must be found better criteria for exchange transfusing newborn with jaundice of unknown aetiology.

As opposed to this, the criteria for exchange transfusing Rhesus sensitised children are satisfactory. Admittedly it is unnecessary in the case of 44 out of every 100 children but, on the other hand, 56 will be saved from death or kernicterus, with the risk of death due to the procedure in only one case.

### Addendum

It has been stated that jaundice of unknown aetiology is a rare finding in premature infants of toxemic mothers (R. Sacrez, J.-M. Lévy, E. Scheppeler & M. Klein: Relations entre l'ictère physiologique du prématuré et la toxémie tardive de la grossesse. *Sem Hôp (Paris) (Ann Péd)* 36: 1219, 1960). Our investigation can neither confirm nor weaken this observation, since only two mothers with toxemia gave birth to premature babies who survived the first week. One infant (1450 g) had its maximum serum bilirubin value (10.4 mg%) on the 4th day and the other

(2400 g) was not jaundiced. As far as the mature babies are concerned our investigation shows that jaundice of unknown aetiology occurs just as frequently in infants of toxemic mothers as in other mature babies. There were 47 mothers with toxemia (both mild and severe) and 11 of their infants had jaundice and serum bilirubin values of  $\geq 10$  mg% (three were exchange transfused). Thus the frequency of jaundice of unknown aetiology was 23.4 % in infants of toxemic mothers and 26.3 % in infants of mothers without toxemia.

### References

1. DYGGVE, H.: Bilirubin studies in premature infants who received menadione derivatives or vitamin K<sub>1</sub> at birth. *Acta Pædiat*, 49: 230, 1960.
2. DYGGVE, H., and MUNK-ANDERSEN, G.: On the incidence of haemolytic disease of the newborn due to ABO incompatibility. *Acta Pædiat*, 49: 249, 1960.
3. HANSEN, E.: Cerebral Palsy in Denmark. Thesis. Munksgaard, Copenhagen, 1960.
4. KILLANDER, A., MÜLLER-EBERHARD, U., and SJÖLIN, S.: Indications for exchange transfusion in newborn infants with hyperbilirubinaemia not due to Rh immunization. *Acta Pædiat*, 49: 377, 1960.
5. KÆRN, T.: Perinatal dødelighed og dens geografiske relationer. Studier over den perinatale dødelighed. IV. *Ugeskr Læger*, 120: 583, 1958.
6. LUNDWALL, F., SØRENSEN, B., and TROLLE, D.: Paper read at the Scand. Congress on Gynaecology. Finland, 1960.
7. NØRREGAARD, S.: Årsagerne til for tidlig fødsel. Thesis. Frost-Hansen, Copenhagen, 1953.
8. PLUM, P.: Etiology of congenital athetosis. *Ann Pædiat Fenn*, 3: 589, 1957.
9. VAUGHAN, V. C., ALLEN, F. H., and DIAMOND, L. K.: Erythroblastosis fetalis. I. Problems in the interpretation of changing mortality in erythroblastosis fetalis. *Pediatrics*, 6: 173, 1950.
10. Erythroblastosis fetalis. IV. Further observations on kernicterus. *Pediatrics*, 6: 706, 1950.

Submitted Dec. 29, 1960  
Accepted Jan. 17, 1961

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## CASE REPORT

# Successful Resuscitation of Two Immature Infants with Cardiac Arrest During Exchange Transfusion

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Exchange transfusion, still the only adequate treatment for hyperbilirubinaemia of infancy, carries a considerable risk to the patient. The overall frequency of complications has been estimated to be as high as 40 % [17] and it has been stated [1] that in more than 3 % of the transfused cases the complication has been severe enough to indicate discontinuation of the transfusion. Diamond emphasizing the importance of optimal technical conditions states that it should be possible to keep the mortality of exchange transfusions to less than 1 % [5].

Fatal accidents in connection with exchange transfusions are in most cases due to circulatory collapse caused by cardiac arrest and both ventricular fibrillation and ventricular asystole have been described [13, 16]. The pathophysiology of these fatal accidents is not well understood, although it has been thoroughly studied [2, 4, 9, 10, 14], and no single parameter has thus far been considered responsible.

The present communication describes two instances of cardiac arrest during exchange transfusion, both successfully treated with cardiac massage after efforts with

the more conventional methods of resuscitation had failed. It is the authors' aim to show that a cardiac arrest in infants does not necessarily mean a fatal outcome and that adequate treatment of these accidents during exchange transfusions may even further decrease the mortality still attendant to the procedure.

### Case Reports

*The first infant* was the fourth offspring of a healthy mother and belonged to blood group A Rh neg. The mother was O Rh pos. Her previous three children were alive and well. The pregnancy, which had been uneventful, terminated at 34 weeks with the birth of an infant weighing 2160 g. The delivery and the immediate neonatal period were normal. On the first day a slight icterus was noted, which had increased on the second day of life. The indirect serum bilirubin was 18 mg/100 ml. Because of the early hyperbilirubinemia, the immaturity of the baby, and the presence of high titers of anti-A antibodies in the mother's blood an exchange transfusion was performed. Four hundred ml of type O red cells in A Rh neg plasma were given through the umbilical vein in lots of 20 ml. During the procedure the child was in very good condition. A second exchange was performed on the third day of life because of an indirect bilirubin of 20 mg/100 ml.

This transfusion was also completed without any difficulties.

On the fourth day of life a third exchange transfusion was performed because the indirect bilirubin level was still at 20 mg/100 ml. When 300 ml had been transfused the infant suddenly, without any previous warning symptoms or signs, gasped 2 or 3 times, and became pale-cyanotic. The transfusion was immediately discontinued and auscultation of the heart revealed no heart sounds. Efforts to initiate heart action were immediately started: the arms and legs were elevated, the head lowered, the thorax was violently compressed antero-posteriorly over the heart, and the precordial area was slapped repeatedly, while oxygen was administered by mask with positive pressure. These procedures were unsuccessful and after approximately 45 seconds a thoracotomy was performed over the heart in the 5th intercostal space. There was virtually no bleeding. The heart was dilated and without apparent contractions.

Without opening the pericardium, cardiac massage was started with the flaccid heart rhythmically pressed between the operator's forefinger and the anterior thoracic wall. After five compressions the tonus of the heart improved and within ten cycles there were spontaneous contractions of the ventricles. Within a few minutes the heart rate was 150 per minute.

The effect of the return of cardiac action was startling: with the first few strokes the color of the skin became pink and the infant started to cry loudly. Endotracheal intubation was not performed for approximately 5 minutes. Despite the fact that the infant was using only one lung until intubation, it nevertheless oxygenated fairly well and was capable of relatively vigorous, intermittent crying.

After cardiac action had resumed the bleeding was controlled, oxytetracycline was instilled into the pleural cavity, the collapsed lung was inflated and the wound was closed in layers. During this procedure a compensatory transfusion of 50 ml blood was given. Continuous suction was applied

to the left pleural space, the child was extubated and started on antibiotics.

The recovery of the infant was entirely uneventful. The pleural suction was discontinued after 24 hours. Electrocardiograms revealed normal standard and precordial leads. Despite repeated and thorough neurological examinations no signs of CNS damage were noted. The serum bilirubin fell steadily. The general appearance and attitude of the child was not different from that of a normal child: she gained weight steadily and was discharged from the hospital on the 20th day of life weighing 2210 g. Follow-up examination at the age of 3 months was entirely normal.

*The second infant*, an immature female twin with a birthweight of 1700 g and belonging to blood group A Rh pos, was delivered subsequent to extraction of its dead twin brother. The delivery terminated an uneventful 33 week pregnancy. The immediate neonatal period was uneventful but on the third day of life she developed icterus with an indirect bilirubin of 10 mg/100 ml, which increased to 21.5 mg/100 ml on the fourth day of life. The child was then admitted to the children's clinic for exchange transfusion. The mother's blood, type O Rh pos, contained anti-A antibodies and an exchange transfusion was performed with O Rh pos rbc in A Rh pos plasma. The infant was in good condition when the transfusion was started, cried vigorously and did not show any signs of circulatory or respiratory disturbances. The transfusion was performed through the umbilical vein and the administered blood was thermostatically heated to 36°.

When the transfusion was almost completed and 220 ml blood had been exchanged, the child suddenly became pale-cyanotic and stopped breathing after 2-3 gasping inspirations. The head was immediately lowered, oxygen was administered by mask with positive intermittent pressure without apparent effect upon the color of the skin or upon the general condition of the child. Auscultation over the heart revealed no heart sounds and efforts to reinstitute regular

heart action were immediately started. The legs and arms of the infant were raised to provide increased venous return to the right heart, vigorous compressions of the thorax over the heart were performed rhythmically, and the precordium was repeatedly beaten while oxygen was given with intermittent positive pressure. When after two minutes these procedures had had no effect upon the child who remained lifeless and pale-cyanotic, a thoracotomy was performed.

The incision was made along the 5th inter-space in the precordium and when the thoracic cavity was incised and the lung had collapsed, the heart was found to be completely dilated, bluish grey in color and without apparent contractions. When the heart was palpated it gave the impression of being completely atonic without vibrations or contractions.

Massage was started with the heart between the operator's forefinger and the anterior thoracic wall. After 10 compressions the tone began to return and these first ten compressions had a dramatic effect upon the general status of the child. The color of the skin improved and the child began to take deep voluntary inspirations. Eye movements returned. There was, however, still no spontaneous heart action seen or felt. At this time endotracheal intubation was attempted unsuccessfully and intermittent positive pressure with oxygen by mask was continued. After five minutes of massage at a rate of 80/min the heart still did not have any contractions of its own. The infant was reacting reasonably normally with efforts to cry and making regular breathing movements with her right lung. Three ml of Ca gluconate was now injected into the right ventricle and massage was continued. When at seven minutes still no spontaneous heart action was apparent, 0.2 mg adrenalin was injected intracardially. After a total of 10 minutes of massage the first heart action was felt, and ventricular contractions at a rate of 20/min started.

Manual massage was continued until the spontaneous heart rhythm at 14 minutes had reached approximately 200/min. The

child now cried vigorously and the cyanosis completely disappeared. Slight bleeding started from the margins of the wound but could easily be controlled. The pediatric surgeon completed the wound closure and established pleural drainage. A transfusion of 20 ml was given with an apparently beneficial effect on the infant, whose skin became more pink. Antibiotics were administered, X-ray of the chest revealed good expansion of the left lung.

The recovery of the patient was remarkably uneventful and the day after the episode the infant was able to drink from the bottle and appeared perfectly well. No signs of CNS damage were observed during the hospital stay that lasted for another two weeks, nor were there any at a follow up examination one month later. Electrocardiographic tracings revealed no signs of myocardial damage.

### Discussion

The authors do not wish to speculate as to the cause of cardiac arrest during exchange transfusions as these problems will be dealt with in a forthcoming communication. The present discussion will be limited to some problems associated with the treatment of the cardiac arrest in infants.

The finding in both the reported cases of a hypotonic dilated heart denotes the presence of ventricular asystole, a situation that in contrast to ventricular fibrillation should be treated only by mechanical stimulation [8]. Although thoracotomy, with intermittent manual compression of the heart must be considered to be the most effective mechanical stimulus and also the most effective circulatory adjuvant in cardiac arrest, the treatment of the asystolic heart has recently been the subject of several communications, report-

ing good results from various procedures not involving thoracotomy. These include methods such as compressing the heart intermittently between the anterior thoracic wall and the spinal column [6, 15], violent compression of the upper part of the abdomen against the diaphragm [11], forceful beating on the precordium [3], and increasing the venous return to the asystolic heart by raising arms and legs of the patient [18]. The mechanical stimulation that a needle, introduced into the myocardium through the thoracic wall creates has been suggested as an activator of spontaneous heart action [7], while injection of drugs such as calcium and adrenalin are supposed to be effective only when the injection is accompanied by effective cardiac massage.

All these methods have in common the fact that they are relatively easy to perform rapidly and do not require operative facilities. The great danger inherent in their application is, however, that valuable and perhaps vital time is lost if they are continued unsuccessfully. This may completely invalidate the beneficial effect of a successful intrathoracic treatment performed later by causing permanent CNS damage to the patient. Only if an adequate circulation can be maintained by extrathoracic methods, as has been reported for the method using thoracic compression [6, 15] is one justified in continuing for some length before thoracotomy is performed. The other methods should be tried quickly, only once, and if unsuccessful be followed by thoracotomy, manual cardiac massage and the administration of adrenalin and calcium [12].

The amazingly good effect upon the

circulation that could be instituted and maintained by the one-finger-massage on the dilated and hypotonic myocardium indicates that the small dimensions of the immature infant do not constitute a contraindication to thoracotomy and this opinion is further supported by the rapidity and ease with which these small infants recovered from the operation. Of particular interest in this connection is the fact, that spontaneous cardiac action could be reconstituted without first establishing an effective ventilation and oxygenation by means of endotracheal intubation together with the finding that both these infants, once they had regained their cerebral function could maintain a, seemingly sufficient, ventilation with only one functioning lung. Both these findings are somewhat unexpected and would indicate that immediate endotracheal intubation is not imperative. This would mean that the immediate problem is one of the return of cardiac action and all efforts should therefore be directed towards that end alone.

It has been the aim of this report to emphasize that thoracotomy may be a lifesaving procedure even in early infancy and should be considered as such and be performed without delay by the physician who recognises the situation, when "external" methods have failed. Whatever the cause of the cardiac arrest may be, it seems reasonable to suspect that cardiac standstill even if not pre-signaled by alarming changes in the general status of the infant, is preceded by electrocardiographic changes. For this reason we would strongly recommend that the ECG be continuously monitored routinely throughout an exchange transfusion.



### Summary

Two instances of asystolic cardiac arrest in immature infants during exchange transfusions are reported. In both cases the collapse came as an unexpected complication at the end of the transfusion. When extrathoracic manipulations to restore spontaneous heart action had been unsuccessfully performed for one and two minutes respectively, thoracotomy was performed and cardiac massage instituted. Spontaneous heart action started in one case shortly after manual compressions

had been instituted and in the other case after 10 minutes of massage and the administration of calcium and adrenalin. The recovery of the infants was completely uneventful and no signs of CNS damage could be detected at the age of two and three months respectively. Methods to institute cardiac action are discussed and it is stressed that thoracotomy and cardiac massage are comparatively easy procedures not contraindicated by the small size of these infants. The use of continuous ECG control during exchange transfusion is recommended.

### References

1. BOGGS, TH. R. and WESTPHAL, M. C.: Mortality of exchange transfusion. *Pediatrics*, 26: 745, 1960.
2. BOLAND, R. P., TRAISMAN, H. S. and PHILIPSBORN, H. F. Jr.: Electrolyte considerations in exchange transfusions for erythroblastosis fetalis. *J Pediat*, 49: 401, 1956.
3. BRANDENBURG, J. T.: Successful treatment by a chest blow of cardiac arrest during myocardial infarction. *JAMA*, 170: 1307, 1959.
4. CAMPBELL, W. A. B.: Potassium levels in exchange transfusion. *Arch Dis Child*, 30: 513, 1955.
5. DIAMOND, L.: Letter to the Editor. *Amer J Dis Child*, 100: 949, 1960.
6. EASTWOOD, N. B.: External cardiac massage. Letter to the Editor. *Lancet*, 1: 564, 1961.
7. Editorial review.: Treatment of cardiac arrest. *Brit Med J*, 1: 409, 1960.
8. Editorial review.: Cardiac arrest and resuscitation. *Lancet*, 2: 1333, 1960.
9. FARQUHAR, J. W. and SMITH, H.: Clinical and biochemical changes during exchange transfusion. *Arch Dis Child*, 33: 142, 1958.
10. GRAHAM, B. D. and HEYN, R. M.: Acid base homeostasis during exchange transfusion of newborn infants with preserved blood. *Pediatrics*, 15: 241, 1955.
11. HEALY, J. B.: External cardiac massage. Letter to the Editor. *Lancet*, 1: 508, 1961.
12. HOSLER, R. M.: Manual on Cardiac Resuscitation. Ed. 2. Ch. C. Thomas Publ., Springfield, Ill. 1959.
13. LEVEEN, H. H., PASTERNAK, H. S., LUSTRIN, I., SHAPIRO, R. B. and NECKER, E.: Hemorrhage and transfusion as the major cause of cardiac arrest. *JAMA*, 173: 770, 1960.
14. MILLER, G., MCCOORD, A. B., JOOS, H. A. and CLAUSEN, S. W.: Studies of serum electrolyte changes during exchange transfusions. *Pediatrics*, 13: 412, 1954.
15. MOLLOY, P. J.: External cardiac massage. Letter to the Editor. *Lancet*, 1: 49, 1961.
16. TAYLOR, W. C., GRIDDALE, CH. B. and STEWART, A.: Unexplained death from exchange transfusion. *J Pediat*, 52: 694, 1958.
17. WALKER, W. and NELIGAN, G.: Exchange transfusions in haemorrhagic disease of the newborn. *Brit Med J*, 1: 681, 1955.
18. WOODWARD, W. W.: Cardiac arrest treated by elevation of the limbs for fifteen seconds. *Lancet*, 2: 1120, 1960.

Submitted March 30, 1961

Accepted April 25, 1961

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CASE REPORT

## Hamman-Rich Syndrome

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In 1935 Hamman & Rich (16) published the first cases of idiopathic, diffuse, interstitial fibrosis of the lungs, but it was not until after 1944 (17) when they described the syndrome again, that an interest in this disease was aroused. Since then about 100 cases have been reported. In 1957 Rubin & Lubliner (34) collected a total of 64 cases, partly from the literature and partly their own. A few of these, however, also suffered from other diseases which might have contributed to the lung disease. A more critical report was published in 1959 by Donohue *et al.* (8), who collected 87 cases from the literature and added 10 cases of their own. Of these 97 cases only 12 were children. The syndrome thus seems to be very rare in childhood, or at least rarely recognized as such.

### Case Report

J. 811/59. Girl, born in 1949 as the second child. The sister is well. No family history of heart or lung diseases. Development was normal. In December 1955 she was operated on for perforated appendicitis. Before operation tachycardia was observed, but no other symptoms of heart or lung diseases. Since the operation she has had increasing cyanosis, loss of appetite, and a tendency to fainting during effort, but no edema, and

no cough. In August 1957 she was admitted to the Pediatric Department at Sundby Hospital.

### Physical Findings

Cyanosis of the lips and dyspnea, slightly enlarged liver, but no edema and no drumstick fingers. BP100/50. Auscultation of the lungs was normal. Auscultation of the heart revealed a gallop rhythm in the 4th left interspace and a faint systolic murmur over the precordium. When readmitted to the hospital in 1958 and 1959, she complained of a tendency to sweating, a feeling of cold feet, headaches and a short dry cough, particularly at night. The condition gradually progressed. From November 1958 edema and ascites were also observed but still there were no signs of drumstick fingers or watch-glass nails. Examination of the heart demonstrated enlargement 2 cm lateral to the mid-clavicular line. A loud, harsh systolic murmur was heard throughout the precordium. Auscultation of the lungs was normal. Death occurred in November 1959.

### Laboratory Examinations 1957

X-ray examination: lungs normal, heart moderately enlarged. Electrocardiogram: right axis deviation. Electrophoresis of serum protein was normal. Blood urea 38 mg %. Arterial oxygen saturation 96%. Erythrocytes 5.69, 6.23, 5.9 million. Leukocytes 16,300, 12,000 and 17,700 mm<sup>3</sup>. Differential

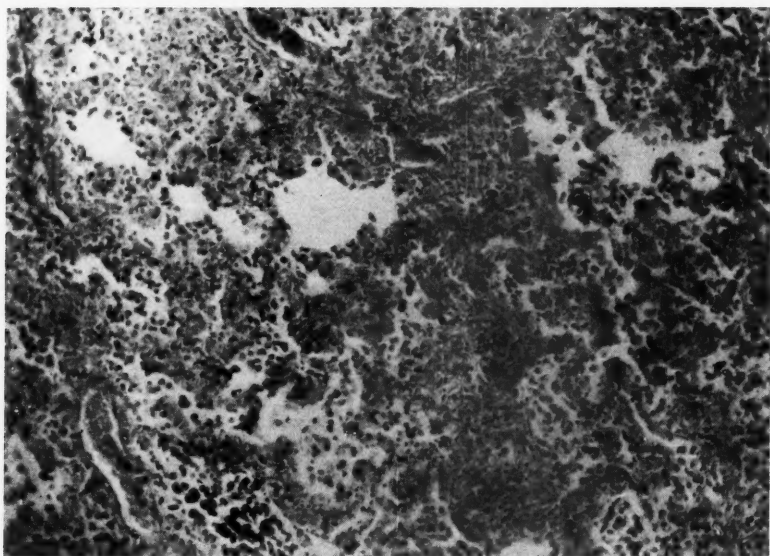


Fig. 1. Microscopy from the lung. Note the diffuse pronounced fibrosis of the alveolar septa (v. Gieson-Hansen:  $\times 73$ ).

count normal. Urine revealed varying proteinuria (maximally 0.2%). Heart catheterization (performed by Ib Boesen, M.D., at Dronning Louises Børnehospital) showed pulmonary hypertension and an increased diastolic pressure in the right auricle and right ventricle, plus low oxygen saturation in the venous blood.

#### September 1959

X-ray examination of the thorax (Fig. 2): lungs normal, heart enlarged with a prominence of the pulmonary arch, the right auricle and the right ventricle. Blood pressure 85 systolic. ECG showed right axis deviation,  $Q_3$  4 mm,  $T_{1-2}$  flat,  $T_3$  isoelectric,  $T_{V2-6}$  negative, qRs pattern in  $V_{2-4}$ , RS pattern in  $V_6$ . Rbc 5.2 million, leukocytes 8040; differential count, neutrophilia. Urine normal, but two days before death proteinuria again appeared. Treatment: digitalis, mercurial diuretics, chlorothiazide and penicillin, without any certain effect. Steroids were not given.

#### Autopsy

This was performed by Hemming Poulsen, M.D. Fifteen hundred ml of serous fluid were found in each pleural sac. The surfaces of the serous membranes were smooth and glossy without fibrinous coating. Lungs: medium-sized, with slight emphysema in both upper lobes, and with slight atelectasis in the inferior lobes. Specimens of the tissue from all pulmonary lobes floated on water. Incision through each pulmonary lobe revealed no macroscopic focal changes, in particular no pneumonic processes. The consistency was not increased and there was no edema. Trachea and bronchi were normal. The pulmonary artery showed slight atheromatosis at the bifurcation, but no changes in the branches. The heart measured  $8 \times 9$  cm and weighed 670 g. The endocardium was normal. The tricuspid valve was dilated and measured 11 cm cut open. Both the right ventricle and the right auricle showed a very high degree of dilatation. The wall of the right ventricle was also markedly hyper-

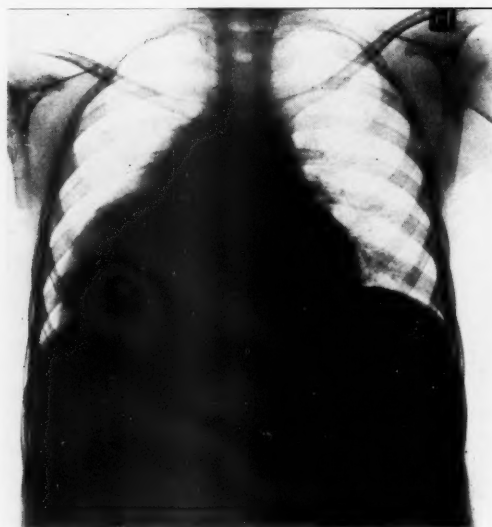


Fig. 2. X-ray of the chest 2 months before death. Note the absence of pulmonary fibrosis. H = right side.

trophic, and measured 7 mm upwards and 11 mm downwards in thickness. The papillary muscles were much thickened. The pulmonary ostium was natural. The left heart was normal. In the other parenchymatous organs there was marked congestion. There was moderate edema of the lower extremities. There was 1000 ml of clear, light ascitic fluid and 150 ml of clear fluid in the pericardium.

*Microscopy of the lung tissue* (Fig. 1). All the lung lobes showed essentially the same histological picture, although the degree of the changes varied considerably. The changes were most pronounced in the lower lobes, and in each of the lung lobes subpleurally. Connective tissue increase was seen in the alveolar walls, at some places with many collagenous fibrils, at other points with many fibroblasts. Dilated, blood-filled capillaries were often observed, whereas there were only a few, small, and dispersed infiltrations of lymphocytes. Granulocytes or plasma cells were not found. The alveolar epithelium was flat to cuboid. There was no edema, but a number of macrophages containing blood

pigments were found in many alveoli. Hyaline membranes were not found. The bronchi showed a slight increase of the connective tissue of the adventitia, but no inflammation. In the branches of the pulmonary artery proliferation of the connective tissue occurred in the adventitia as well as in the muscularis mucosa. The intima was within normal limits. There were no thromboses nor muscular hyperplasia. Elastic tissue staining (11) showed no abnormalities. Microscopy of the heart revealed rather pronounced fibrosis of the myocardium. No inflammatory cells were present. Microscopy of cerebrum demonstrated edema and acute and chronic anoxic changes.

### Discussion

Clinically, Hamman-Rich syndrome is characterized by cough, fever, loss of weight, tendency to perspiration, cyanosis and dyspnea (34), all of which are nonspecific symptoms of heart and lung disease. X-ray examination of the thorax

TABLE 1. *Previously described cases of Hamman-Rich syndrome in children verified by lung microscopy.*

		Sex	Age at death
Luneth <i>et al.</i>	(1955)	F	11 weeks
Bradley	(1956)	F	9 years
Grant <i>et al.</i>	(1956)	F	13 years
Donohue	(1956)	F	1½ years
		F	7 weeks
Aranson	(1956)	M	9 years
Obracaj <i>et al.</i>	(1956)	F	?
		F	3 years
		F	8 years
Baar <i>et al.</i>	(1957)	M	3 years
Feinerman <i>et al.</i>	(1957)	M	?
		F	7 months
Donohue <i>et al.</i>	(1959)	F	6 months
		F	1 year
		F	8 months
Mann	(1959)	F	8 months
Wilson <i>et al.</i>	(1960)	M	3 months
		F	1 month
+ 2 cases, not described			

thickening of the alveolar septa because of proliferation of fibroblasts, dilatation and proliferation of capillaries, and edema. Sometimes hyaline membranes are seen. Either no or only slight organization of alveolar exudate is seen. The vessels are often moderately hypertrophic because of hyperplasia of the intima or media (14, 15, 17, 32).

As has been stated previously, the etiology is unknown. Fibrosis of the lungs as such is a non-specific reaction (38) which may be caused by numerous agents (6), including infections, inhalation, systemic diseases, circulatory disturbances and irradiation. Some authors, for example, Baar *et al.* (2), mention collagenosis as an etiologic possibility, but the microscopic picture of the lung tissue in collagen diseases seems, however, to be slightly different from what is seen in the Hamman-Rich syndrome (10, 23, 27, 33). Several authors, among others Pokorny *et al.* (32), believe the cause to be due to insufficient resolution following one or more attacks of interstitial pneumonia because of a defect in fibrinolysis. Proof of this theory is still lacking. Other authors mention the possibility of a hereditary disposition (24, 38) or a hereditary congenital defect of lung tissue (8). Of the reported cases about 25% have had a familial occurrence. Congenital dysgenesis of the lung tissue may well explain the cases in infants.

In Table 1 are listed the previously reported cases in children in which microscopic examination of the lung tissue is reported. Donohue's two cases (7) and the case published by Luneth *et al.* (22) were previously described as interstitial plasma cell pneumonia, but Donohue *et al.* (8) later revised these diagnoses when no pneumo-

shows varying, non-specific lung infiltrations (6, 20, 30, 34), or may even be normal (19, 31). Cor pulmonale is seen in about 50% of the cases (30). The laboratory tests do not give any definite support to the diagnosis (20, 34). Cardiac catheterization shows increased pressure in the right ventricle and pulmonary artery and reduced arterial oxygen saturation (36). In our case the pressure was increased but the arterial oxygen saturation was normal and there was a large arterio-venous oxygen difference as is seen in primary pulmonary arterial hypertension (9).

Only microscopic examination of the lung tissue can give the correct diagnosis (6, 30, 32). The microscopic picture is first and foremost characterized by a diffuse interstitial increase of connective tissue, metaplasia of the alveolar epithelium cells,

cystis carinii were isolated, and now consider them a form of pulmonary dysgenesis. Wilson *et al.* (39) state that the microscopic picture in their four cases in prematures is quite like the picture in Hamman-Rich syndrome, but all the same they call them "a new form of respiratory disease" as no cases have been reported in the neonatal period. This point of view is hardly correct, as there are descriptions of cases in children in all age groups including early infancy. The existence in the neonatal period fits well with the theory advanced by Donohue *et al.* of congenital pulmonary dysgenesis (8).

In addition to the cases listed in the table, there are other cases in the literature where the diagnosis is more uncertain or even doubtful. This is true about Březina's four cases (4), one of the cases described by Donohue *et al.* (8), and about Levinský's case (21), because microscopic examination of the lung tissue was not performed in these cases. The case reported by Marie *et al.* (26) differs both macroscopically and microscopically from the usual picture, but may be considered as a variant of Hamman-Rich syndrome. On the other hand, the diagnosis in Diamond's case (5) is hardly correct, as has also been pointed out by Donohue *et al.* (8). In addition to fibrosis of the lungs Diamond found rather pronounced changes in the vessels, which may have been the primary cause of the lung disease.

Of the certain cases listed in Table 1, and our own case, 15 are girls and only four are boys. This does not correspond to the distribution found among adults, where the number of cases among men and women is equal (8). The unequal distribution must, however, be evaluated with some caution because of the few cases reported among children.

In some cases treatment with cortisone

has shown to have a good effect; most often it has only given subjective improvement (12, 13, 18, 20, 37), but in a few cases an improvement in the X-ray appearance of the lungs has been reported (29, 31, 35). On discontinuation of the treatment death has occurred a few days later in some cases, for which reason it must be recommended that treatment be continued without any regard to a lacking effect (20, 29, 35). In order to achieve an effect with cortisone, it is important to start treatment as early as possible in the course of the disease, since steroids can only delay the proliferation of the fibroblasts, and are without effect once fibrous tissue is formed (20, 29, 31).

Without cortisone the prognosis is bad, but the duration of the disease may vary from weeks to many years (8). It may be possible to improve the prognosis somewhat with cortisone treatment (31, 37), but the observations to date are too few and of too short duration to permit a final evaluation.

### Summary

A case of the Hamman-Rich syndrome in a 10 year old girl is reported. The duration of the disease was 5 years. Microscopy of the lung tissue showed an increase of connective tissue and dilated capillaries in the alveolar walls. Treatment with digitalis, penicillin and diuretics were without effect. A survey of previously reported cases of this syndrome in children is given.

## References

1. ARANSON, A.: Hamman-Rich syndrome. *J Maine Med Ass*, 47: 105, 1956.
2. BAAR, H. S. and BRAID, F.: Diffuse progressive interstitial fibrosis of the lungs in childhood. *Arch Dis Child*, 32: 199, 1957.
3. BRADLEY, C. A.: Diffuse interstitial fibrosis of the lungs in children. *J Pediat*, 48: 442, 1956.
4. BREZINA, Z.: Über Lungenfibrose im Kindesalter. *Kinderärztl Prax*, 27: 57, 1959.
5. DIAMOND, I.: The Hamman-Rich syndrome in childhood; report of a case with unilateral pulmonary arterial and venous stenosis and atriovenous occlusion. *Pediatrics*, 22: 279, 1958.
6. DONOGHUE, F. E. and WOOLNER, L. B.: Pulmonary fibrosis. *Med Clin N Amer*, 1909, 1954.
7. DONOHUE, W. L.: Interstitial plasma cell pneumonia. *Lab Invest*, 5: 97, 1956.
8. DONOHUE, W. L., LASKI, B., UCHIDA, I. and MUNN, J. D.: Familial fibrocystic pulmonary dysplasia and its relation to the Hamman-Rich syndrome. *Pediatrics*, 24: 786, 1959.
9. DRESDALE, D. T., SCHULTZ, M. and MICHOTOM, R. J.: Primary pulmonary hypertension. *Amer J Med*, 11: 686, 1951.
10. ELLMAN, P. and CUDKOWICZ, L.: Pulmonary manifestations in the diffuse collagen diseases. *Thorax*, 9: 46, 1954.
11. ESKELUND, V.: Fast two-step trichrome staining for paraffin and frozen sections combining demonstration of elastic fibres, fibrin and mucus with v. Gieson stain. *Acta Path Microbiol Scand*, 40: 105, 1957.
12. FEINERMAN, B. and HARRIS, L. E.: Unusual interstitial pneumonitis. *Proc Mayo Clin*, 32: 637, 1957.
13. FREEDMAN, A. and DEATON, W. T.: Diffuse interstitial fibrosis of the lungs (Hamman-Rich syndrome). Successful palliation of a case with steroid therapy. *Dis Chest*, 34: 557, 1958.
14. GOLDEN, A. and BRONK, T. T.: Diffuse interstitial fibrosis of lungs. *Arch Int Med*, 92: 606, 1953.
15. GRANT, I. W. B., HILLIS, B. R. and DAVIDSON, J.: Diffuse interstitial fibrosis of the lungs (Hamman-Rich syndrome). *Amer Rev Tuberc*, 74: 485, 1956.
16. HAMMAN, L. and RICH, A. R.: Fulminating diffuse interstitial fibrosis of the lungs. *Trans Amer Clin Climat Ass*, 51: 154, 1935.
17. HAMMAN, L. and RICH, A. R.: Acute diffuse interstitial fibrosis of the lungs. *Bull Johns Hopkins Hosp*, 74: 177, 1944.
18. HARRIS, M. S., PREUSS, F. S., GOLDMAN, A. and FRIEDMAN, N.: Cortisone therapy in Hamman-Rich syndrome. *Amer Rev Tuberc*, 76: 123, 1957.
19. HEPPLESTON, A. G.: Chronic diffuse interstitial fibrosis of the lungs. *Thorax*, 6: 426, 1951.
20. HOFF, H. R.: The Hamman-Rich syndrome. *New Engl J Med*, 259: 81, 1958.
21. LEVINSKÝ, L.: Chronická difúzní intersticiální fibrosa plic (idiopathická). *Čas Lék Čes*, 92: 976, 1953.
22. LUNSETH, J. H., KIRMSE, T. W., PREZYNA, A. P. and GERTH, R. E.: Interstitial plasma cell pneumonia. *J Pediat*, 46: 137, 1955.
23. LUSTOK, M. J. and KUZMA, J. F.: Rheumatic fever pneumonitis: A clinical and pathologic study of 35 cases. *Ann Int Med*, 44: 337, 1956.
24. MACMILLAN, J. M.: Familial pulmonary fibrosis. *Dis Chest*, 20: 426, 1951.
25. MANN, T. P.: Diffuse progressive interstitial fibrosis of lungs in infancy. *Proc Roy Soc Med*, 52: 638, 1959.
26. MARIE, J., HENNEQUET, A., HÉBERT, S. et BOCQUET, L.: Sur une variété particulière de fibrose pulmonaire subaiguë de l'enfant, avec aspect cérébroïde de la surface des poumons. *Sem Hôp Paris*, 33: 4018, 1957.
27. MCGEEHEE HARVEY, A., SHULMAN, L. E., TUMULTY, P. A., CONLEY, C. L. and SCHOENRICH, E. H.: Systemic lupus erythematosus: Review of the literature and clinical analysis of 138 cases. *Medicine (Balt)*, 33: 291, 1954.
28. OBRACAJ, W. and NEORAL, L.: Postępująca śródmiąższowa marskość płuc wieku dziecięcego. *Pediatr Pol*, 31: 509, 1956.
29. PEABODY, J. W., BUECHNER, H. A. and ANDERSON, A. E.: Hamman-Rich syndrome. *Arch Int Med*, 92: 806, 1953.
30. PEABODY, J. W. and PEABODY, J. W., Jr.: Diffuse fibrosing interstitial pneumonitis (Hamman-Rich syndrome). *Dis Chest*, 26: 709, 1954.
31. PINNEY, C. T. and HARRIS, H. W.: Hamman-Rich syndrome. *Amer J Med*, 20: 308, 1956.
32. POKORNY, C. and HELLWIG, C. A.: Diffuse interstitial fibrosis of the lungs. *Arch Path*, 59: 382, 1955.
33. PRICE, T. M. L. and SKELTON, M. O.: Rheumatoid arthritis with lung lesions. *Thorax*, 11: 234, 1956.
34. RUBIN, E. H. and LUBLINER, R.: The Hamman-Rich syndrome: Review of the literature and analysis of 15 cases. *Medicine (Balt)*, 36: 397, 1957.
35. SCHECHTER, M. M.: Diffuse interstitial

- fibrosis of the lungs. *Amer Rev Tuberc*, 68: 603, 1953.
36. SILVERMAN, J. J. and TALBOT, T. J.: Diffuse interstitial pulmonary fibrosis camouflaged by hypermetabolism and cardiac failure: Ante-mortem diagnosis with biopsy and catheterization studies. *Ann Int Med*, 38: 1326, 1953.
37. VAN SLYCK, E. J.: Diffuse interstitial pulmonary fibrosis (Hamman-Rich syndrome): Diagnosis by lung biopsy; treated with cortico-steroids. *Dis Chest*, 31: 593, 1957.
38. WILDBERGER, H. L. and BARCLAY, W. R.: Diffuse interstitial pulmonary fibrosis. *Ann Int Med*, 43: 1127, 1955.
39. WILSON, M. G. and MIKITY, V. G.: A new form of respiratory disease in prematures. *Amer J Dis Child*, 99: 489, 1960.

Submitted Nov. 11, 1960

Accepted Nov. 25, 1960

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## CASE REPORT

# Goitrous Hypothyroidism Simulating Thyrotoxicosis

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The following case is reported in order to draw attention to diagnostic difficulties in the case of a boy who developed a goitre and was said to be unduly nervous. The results of medical treatment, once the diagnosis of hypothyroidism was established, were impressive.

### Case report

A boy of 8 was admitted to another hospital in November, 1954, with a month's history of swelling of the neck. His maternal cousin and aunt were said to have had similar swellings and partial thyroidectomy had been performed in each case. The family diet did not suggest a deficiency of iodine. The early life of the patient had been uneventful and he had passed his milestones normally. He was of average intelligence. He was found to have a radio-iodine uptake of 90 per cent with a 24-hour excretion of 10 per cent, which was considered in favour of thyrotoxicosis.

He was treated with iodine for a month without improvement; the dose was doubled for a further month, but again there was no improvement. He was then given phenobarbitone, which was stopped when he became very excitable. In July, 1955, he began a two-month course of propylthiouracil 50 mg three times daily. His goitre and nervousness increased. A thyroid murmur was said to have been heard and he had a tremor and moist palms, but no eye signs. There was no

history of weight loss and bowel action was normal. It was decided to refer him for another opinion as a case of thyrotoxicosis not responding to treatment.

Antithyroid treatment was stopped at the time of admission to the Hospital for Sick Children in September, 1955, when he had a smooth, moderately firm goitre with a neck circumference of 34.8 cm (Figure 1). There was no tremor or sweating. B.P. 100/70. Pulse 80-110. Sleeping pulse 60-90.

*Investigations.* Serum cholesterol 232 mg/100 ml. B.M.R. 103 per cent of normal for height and weight and 116 per cent for age. His bone age was 8 years.

It was concluded that there was no evidence of thyrotoxicosis and that he might be suffering from an enzyme defect which impaired his ability to synthesize thyroid hormone. He was discharged to be observed without treatment.

He attended monthly in the next five months when his neck appeared to decrease in size. He grew and gained weight slowly.

He was readmitted in April, 1956, for reassessment. His neck circumference had decreased by 3.2 cm since his previous admission, but there were some nodules palpable at the right apex of the thyroid. There was no tremor or sweating. B.P. 120/80. Pulse 80-120.

*Investigations.* Serum cholesterol 120 mg/100 ml. Haemoglobin 86 per cent.

It was concluded that no treatment was indicated and he was discharged.

During the next six months he developed a tremor and moist palms. He gained 1.35

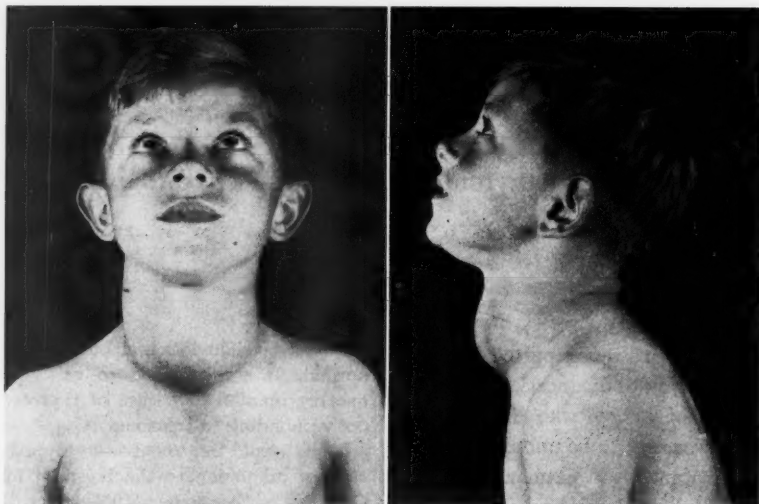


Fig. 1. Patient in 1955, aged 9, after 10 months of antithyroid treatment.

kg in weight, but was reported to be excitable. He was readmitted in December, 1956, for further investigation. His neck circumference had increased to 34 cm and there was no change in the consistency of his thyroid gland. He still had a tremor and moist palms. No thyroid murmur was heard and there were no eye signs. B.P. 110/80. Pulse 90-120. Sleeping pulse 70-90.

*Investigations.* Haemoglobin 94 per cent. Serum cholesterol 185 mg/100 ml. B.M.R. 128 per cent of normal for height and weight and 139 per cent for age. Chest X-ray showed normal lung fields. There was a soft tissue swelling in the neck and the trachea was displaced and compressed. Because of the raised B.M.R. he was thought to be thyrotoxic.

Antithyroid treatment was restarted with potassium perchlorate 200 mg twice daily on the 8th January and, on the 22nd January, thyroid 8 mg twice daily was added, and he was discharged on this therapy. On the 26th January his B.M.R. was 112 per cent of normal for height, weight and age. By discharge on the 29th January, his neck circumference was 33 cm.

In March, his neck circumference had increased to 35.5 cm and he still had tremor and moist palms. His voice had become hoarse. There were no eye signs. The potassium perchlorate was stopped and the thyroid continued for a further two weeks. Six weeks later there was no change and he was put on Lugol's iodine 0.3 ml three times daily, which was increased by 0.06 ml daily to 0.6 ml three times daily. He was unchanged again in September and the Lugol's iodine was reduced to 0.18 ml twice daily. In November, his neck circumference had increased to 36.8 cm and treatment was stopped. By July, 1958, his neck circumference was 36.5 cm. Since his discharge he had gained weight steadily. He was started on thyroid 32.5 mg three times daily in an attempt to suppress his T.S.H. (thyroid stimulating hormone). A month later his neck circumference was 34 cm and he had gained almost 0.5 kg in weight. In October, his neck circumference was 33 cm and his pulse 84. The improvement in his goitre was maintained when he was seen in March, 1959. His pulse was 88 and he had gained 0.9 kg in six months. He was not sweating.

It was felt that this response to thyroid was good evidence that he was unable to manufacture thyroid hormone in adequate quantities and that his goitre resulted from excessive T.S.H. It was decided to stop all treatment and to readmit him at least three months later for investigations to settle this point.

He was admitted in September, 1959, aged 13, and was found to be a calm, cheerful, co-operative boy. Bowel action had been normal and there had been no symptoms which might have been attributed to obstruction due to his enlarged thyroid gland. He was in a Secondary Modern School where his educational attainments were satisfactory. His neck circumference was 36.2 cm. He was below the 10th percentile for height and below the 25th for weight. His colour was satisfactory. B.P. 126/80. Pulse and sleeping pulse 80-110. Apart from some nodules at the right apex of his thyroid, there was uniform soft enlargement. No thyroid murmur was heard and he did not have eye signs. His palms were moist, but not warm. There was no tremor. His skin was of normal texture. There was no sign of secondary sex characters.

**Investigations.** Haemoglobin 94 per cent. Serum cholesterol 175 mg/100 ml. Calcium 10.1 mg/100 ml. Phosphorus 4.0 mg/100 ml. Alkaline phosphatase 16 units/100 ml. Blood sugar curve, fasting was 105 mg/100 ml,  $\frac{1}{2}$  hour — 142 mg/100 ml, 1 hour — 128 mg/100 ml,  $1\frac{1}{2}$  hours — 121 mg/100 ml, 2 hours — 116 mg/100 ml,  $2\frac{1}{2}$  hours 116 mg/100 ml. Twenty-four-hour urinary excretion of creatinine 875 mg and of calcium 88 mg. B.M.R. 139 per cent of normal for age, 105 per cent when referred to creatinine standards of Talbot *et al.* (8). X-ray of wrists for bone age 11-12 years. P.B.I. 2.9 mg/100 ml. Radio-iodine uptake 91 per cent in  $5\frac{1}{2}$  hours, radio-activity not discharged by oral potassium perchlorate (400 mg). Renal iodide clearance using radio-iodine 32 ml/min. (Normal 30-35 ml/min.) The following stable iodine estimations were performed by Dr. E. Shalom of University College Hospital Medical School using an unpublished tech-

nique. Butanol extractable iodine (B.E.I.) 3.3 mg per cent. Plasma iodine 1.3 microg per cent (0.2 microg per cent lower limit of normal). Urine iodide 93 microg/24 hours. (Normal 100-150 microg/24 hours.) The latter two samples were taken at different times and were, therefore, unsuitable for calculating the renal iodide clearance. Urine organic iodine 8 microg/24 hours. (Normal up to 10 per cent of total iodide.) Plasma iodotyrosines and butanol non-extractable iodine not detected.

The P.B.I. and the B.E.I. performed in different laboratories were subnormal, thereby confirming the suspicion of hypothyroidism. (The former was 0.4 mg per cent less than the latter, which is theoretically impossible, but the difference is within the limits of usual variability.) He was restarted on thyroid on the 2nd of October and this was increased until he was receiving 260 mg daily by the 20th of October. He was discharged on this dose when his neck circumference was already reduced to 33.6 cm.

In March, 1960, his neck measurement was 33 cm (Figure 2). He had gained 1.35 kg and grown 2.9 cm since restarting thyroid.

### Discussion

Four specific defects in the utilization of iodine for the formation of thyroxine had been distinguished by Stanbury *et al.* (1,7). All are characterized by a high and rapid iodine uptake, which is unaffected in the latter three groups by an oral dose of potassium perchlorate.

#### a) Failure to Organify Iodine

These patients are unable to combine iodine with organic molecules so that a large dose of potassium perchlorate causes the immediate release of iodine from the gland. Direct assay on thyroid biopsies in such a case has confirmed the presence of inorganic iodine (6). Stanbury suggests that the defect is an absence of oxidase in

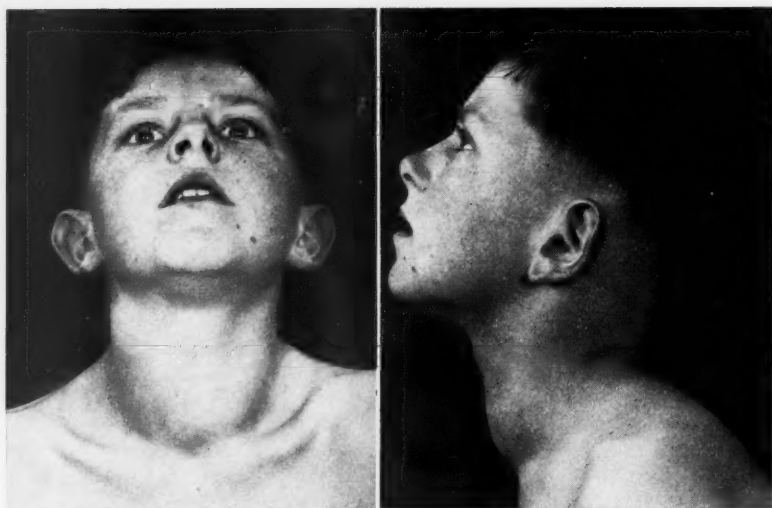


Fig. 2. Patient aged 13, five months after starting 260 thyroid daily.

the thyroid cells. However, Haddad & Sidbury (3) consider that an iodine defect and an abnormal receptor protein might produce the same picture.

b) *Failure of De-iodination of Iodotyrosines*

Absence of de-iodinase both in the thyroid and the peripheral tissues leads to the escape of mono and di-iodotyrosines into the blood and from the body. The hypothyroid state is considered to result from the loss of these hormonal precursors (7).

This concept of a pure de-iodinase defect producing hypothyroidism has been criticised by Gardner *et al.* (2) who suggest that there must be an associated defect in synthesis or release of thyroxine.

c) *Failure of Coupling of Iodotyrosines*

Stanbury *et al.* (6) describes a patient in whom thyroxine is found in the blood and

thyroid gland in subnormal quantities. Large quantities of mono and di-iodotyrosines are found in a thyroid biopsy. Werner *et al.* (9) also feel that a failure of coupling may explain the defect in hormone synthesis in their case where they found large quantities of mono and di-iodotyrosines in the blood and thyroid gland with normal de-iodinase activity of thyroidal and peripheral tissues.

d) *Formation of Abnormal Iodoproteins*

The formation of metabolically inactive iodinated proteins may be associated with hypothyroidism (1). In such cases the P.B.I. may be high and it is the B.E.I. which is the more reliable index of the thyroid state.

In the present case, the data available enables three of these four defects to be confidently excluded. The gland radioactivity at the height of its uptake was not discharged by an oral dose of potas-

sium perchlorate (400 mg) as would have been the case if there was a defect in the organification or iodine. The fate of labelled iodotyrosine was not determined, but the failure to detect iodotyrosine in the plasma and the urinary excretion of normal amounts of iodinated organic components makes the de-iodinase type of defect very unlikely. Iodine was not detected in the plasma fraction which was protein precipitable, but not butanol extractable and this excludes the defects associated with abnormal iodoprotein synthesis.

The possibility remains that this case may be similar to that described by Stanbury *et al.* (6) where the defect was attributed to a failure of coupling of iodotyrosines. The similarity would be established by demonstrating a raised iodotyrosine content of a thyroid biopsy, but biopsy was not undertaken. Certainly the case differs from that of Werner *et al.* (9) in that iodotyrosines were not found in the plasma.

The majority of cases of enzyme defects in the synthesis of thyroid hormone, which have been reported, have been characterized by obvious clinical features of cretinism. Though the case reported here would be classified as a cretin by Stanbury (7) using as his definition "permanent retardation in the development of the skeleton or C.N.S. resulting from thyroid deficiency which existed during foetal or early neonatal life", it possessed few of the clinical features of cretinism and, in such a case, the description goitrous hypothyroidism is more helpful to clinicians than familial or sporadic cretinism.

That this case was regarded for four years as one of thyrotoxicosis was partly

due to an apparently raised B.M.R., illustrating the unreliability in childhood of B.M.R.s related to age. Use of the creatinine standards of Talbot *et al.* (8) led to the calculation of a B.M.R. which was a more accurate index of the thyroid state. A reminder is provided that thyrotoxicosis is not the sole cause of a raised radio-iodine uptake.

The results of the treatment of the goitre have been very satisfactory and this is undoubtedly due to the large doses of thyroid which have been used. Thyroid surgery may not now be required for cosmetic purposes, but this would be indicated in the event of any increase in the size of the apical thyroid nodules, because of the possibility of malignant change. Hubble used 200 mg of thyroid daily in treating a male patient of the same age and had equally good results, but the patient of Stanbury *et al.* (6) was treated intermittently in her teens with smaller doses of thyroid, viz. 100 mg and her thyroid enlarged progressively, presumably due to failure to remove the stimulus to T.S.H. production. In March, 1960, his neck measurement was 33 cm (Fig. 2). He had gained 1.35 kg and grown 2.9 cm since restarting thyroid. This weight gain and growth was maintained when he was seen in December 1960. His neck then measured 32.4 cm.

### Summary

A case of goitrous hypothyroidism in a boy of 13 is described which has been successfully treated with 260 mg of thyroid daily. The defect in this case may have been due to a failure in the coupling of iodotyrosines. For four years the patient

was regarded as suffering from thyrotoxicosis. This was largely due to the association of goitre with nervous manifestations, viz. excitability, tremor and moist palms. An incorrectly interpreted radio-iodine uptake and an apparently raised B.M.R. were thought to support the diagnosis. Indications of the true diagnosis were the family history of goitre without iodine deficiency, the retarded physical development and bone age, the normal temperature of the palms, the failure to confirm the presence of the thyroid murmur, the absence of eye signs, the discrepancy between the sleeping and the waking pulse,

the failure to respond antithyroid treatment and the good response to treatment with thyroid 30 mg three times daily before the diagnosis was established. The finding of a low P.B.I. clinched the diagnosis of hypothyroidism.

### Acknowledgements

My thanks are due to Sir Wilfrid Sheldon for his advice and permission to publish this case: To Dr. E. E. Pochin for his criticism and for the radio-iodine estimations; To Dr. E. Shalom who performed most of the stable iodine estimations: And to Mr. D. Martin for the photographs.

### References

1. DE GROOT, L. J., POSTEL, S., LITVAK, J. and STANBURY, J. B.: Peptide-linked iodotyrosines and iodothyronines in the blood of a patient with congenital goitre. *J Clin Endocr*, 18: 158, 1958.
2. GARDNER, J. U., HAYLES, A. B., WOOLNER, L. B. and OWEN, C. A.: Iodine metabolism in goitrous cretins. *J Clin Endocr*, 19: 638, 1959.
3. HADDAD, H. M. and SIDBURY, J. B.: Defect of iodinating system in congenital goitrous cretinism. *J Clin Endocr*, 19: 1446, 1959.
4. HUBBLE, D.: "Familial cretinism." *Lancet*, I, 1112, 1953.
5. QUERIDO, A., STANBURY, J. B., KASSENAAAR, A. A. H. and MELJER, J. W. A.: The metabolism of iodotyrosines. *J Clin Endocr*, 16, 1096, 1956.
6. STANBURY, J. B., KALERVO OHELA and PITT RIVERS, R.: The metabolism of iodine in two goitrous cretins compared with that in two patients receiving methimazole. *J Clin Endocr*, 15, 54, 1955.
7. STANBURY, J. B. and QUERIDO, A.: Genetic and environmental factors in cretinism: A classification. *J Clin Endocr*, 16, 1522, 1956.
8. TALBOT, N. B., WORCESTER, J. and STEWART, A.: New creatinine standard for basal metabolism and its clinical implication. *Amer J Dis Child*, 58, 506, 1939.
9. WERNER, S. C., BLOCK, R. J., MANDL, R. H. and KASSENAAAR, A. A. H.: Pathogenesis of a case of congenital goitre with abnormally high levels of S.P.I. and with mono and di-iodotyrosine in the serum. *J Clin Endocr*, 17, 817, 1957.

Submitted Oct. 26, 1960

Accepted Nov. 16, 1960

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England



## CASE REPORT

## Leydig Cell Tumour of the Testis in a 6½ Year Old Boy

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Leydig cell tumour is a rare condition, especially in children. About 30 cases have hitherto been reported. Bishop *et al.* have recently reviewed 26 cases (1). Savard *et al.*, in March 1960 (4), reported a case of virilizing tumour in the testis. The tumour had the morphologic appearance of a Leydig cell tumour, but biochemical activities were suggestive of an adrenocortical origin. In children the tumour is invariably associated with precocious masculinization. In the present paper the condition is discussed in connection with a case report and with particular reference to urinary steroid excretion.

## Case Report

A boy aged 6½ years (Hospital records U.H. 9801) was admitted to the Paediatric Department of Rikshospitalet in January 1960 because of precocious puberty. There had been no known endocrine disturbance in his family. He was the product of an uncomplicated delivery at term and weighed 3300 g, and measured 53 cm. He developed normally to the age of 2 years. When he started to talk, he was noted to have an unusually deep voice and the voice became even deeper as time proceeded. From the age of 3 years he began to grow unusually rapidly and in particular his penis became larger than normal. Pubic and axillary hair was first noted when he was 4 years old,

and at the same time he exhibited acneiform eruptions. There have been no known erections or emissions.

Physical examination on admission revealed a boy with the appearance of a muscular and hirsute youth (Fig. 1). His height of 146 cm was at the 90th percentile for 10 years of age. He had short lower extremities, measuring from the top of the symphysis to the heels 71 cm, with an upper/lower segment ratio of 1.06 (the average ratio for an 8 year old boy). His weight of 45.9 kg was at the 97.5th percentile for his height. There were pronounced acneiform eruptions, the voice was deep and he had extensive growth of pubic and axillary hair.

The penis was of adult size and proportion, measuring in the nonerectile state 11 cm. The left testis was enlarged, measuring 3 × 2 cm, with a localized firm nodule in the posterior part. The right testis was small, measuring 2 × 1 cm. The blood pressure was 100/70. The patient's mental capacity corresponded to his chronological age, but he was rather shy and reserved.

*Laboratory findings:* The concentration of Hb. was 14.7 g/100 ml, the leucocyte count 6200 mm<sup>3</sup> with 43 percent neutrophils, 4 band forms, 2 monocytes and 51 lymphocytes. Erythrocyte sedimentation rate was 5 mm/h. Repeated routine urinalyses were normal. The serum concentration of potassium, sodium and nitrogen urea were also normal. The results of hormone analyses on 24 hours urine are shown in the table. Roentgenograms of the skull, chest and skeletal system were normal, except for a bone





Fig. 1. The patient prior to the operation together with a normal boy of the same age.

age corresponding to about 17 years. Pyelograms were normal and there were no roentgenologic signs of adrenal tumour. An electroencephalogram was normal. The administration of 100 mg cortisone daily for ten days did not diminish the size of the left testis, and had no influence on the amount of 17-ketosteroids in the urine. A biopsy from the firm nodule in the left testis revealed a considerable proliferation of interstitial cells, interpreted by the pathologist as Leydig cell tumour or hyperplasia.

Based on the above, the patient was considered to have a Leydig cell tumour and an extirpation was performed February 1, 1960. During the operation a biopsy specimen was taken from the right testis.

The gross specimen revealed a testis measuring  $3.5 \times 2.5 \times 2$  cm. The cut surface showed a central area of grayish-brown colour, with a sharp boundary to normal testis parenchyma (Fig. 2). Microscopically the central area consisted of relatively uniform, large polyhedral cells with eosinophilic granular cytoplasm containing lipoid vacuoles. There were no crystalloids present. The nuclei were round, slightly eccentric and regular. The tumour had no capsule, but was clearly demarcated. Scattered atrophic testi-

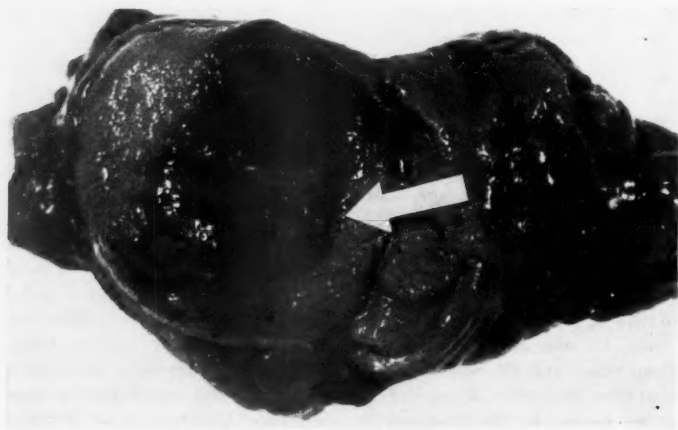


Fig. 2. The gross specimen showing the cut surface of the testis, with a central, grayish-brown area and the arrow pointing to the borderline between this and normal testicular parenchyma. The epididymis and the spermatic cord to the right.

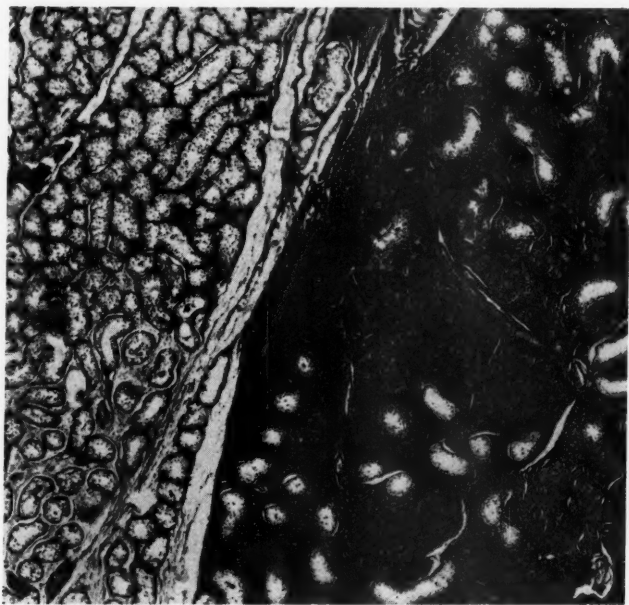


Fig. 3. Low magnification picture showing the clearly demarcated tumour tissue to the right with scattered atrophic testicular tubules in the tumour-parenchyma. Normal testis tissue to the left.

ular tubules were seen in the tumour parenchyma.

The testicular tissue outside the tumour showed normal tubules with spermatogonies, spermatocytes, but no mature sperms. Interstitial cells were neither observed in these areas nor in the biopsy from the right testis.

*Follow up:* Three months later, in May 1960, he was re-admitted for follow up. There were no demonstrable alteration in his signs of precocity. The right testis had grown remarkably in size, measuring  $4 \times 2$  cm.

### Discussion

Cases of precocious sexual development may be classified as complete or incomplete. *The complete type* involves an increased amount of gonadotrophins. The

sex hormones are secreted in amounts normal for adolescents and in boys the testes are fully matured. Diseases of the central nervous system are the underlying cause of this condition.

In cases of *the incomplete type* there is precocious development of secondary sex characters without increased amounts of gonadotrophins. Diseases of the adrenal cortex or of the gonads are the cause.

The clinical picture in the present case together with the fact that the gonadotrophins in all but one analysis were less than 6 units per day permitted classification of the patient as an incomplete type of precocious sexual development (macrogenitosomia praecox).

Accordingly the possible diagnoses were

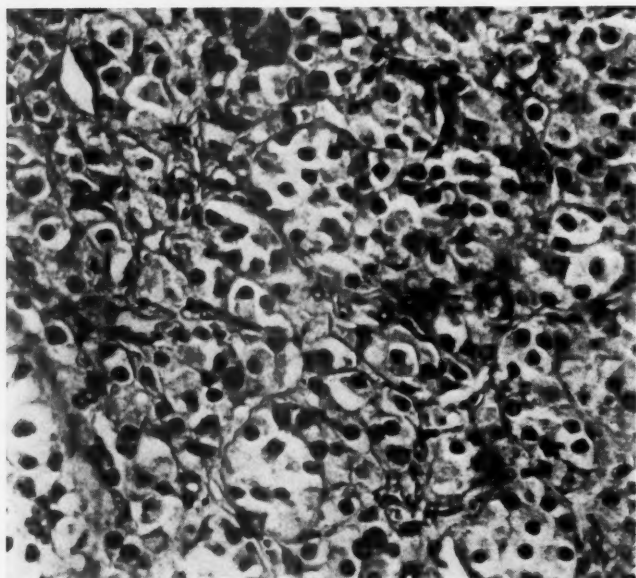


Fig. 4. High magnification view of the tumour: large, uniform and polyhedral cells with granular, eosinophilic cytoplasm and lipid vacuoles along with round, slightly eccentric and regular nuclei.

either adrenocortical hyperfunction (tumour or hyperplasia) or interstitial cell hyperfunction. (Leydig cell tumour or hyperplasia). The patient had an enlarged left testis and there were no roentgenologic evidence of tumour in the adrenals. The enlargement of the left testis might then depend on interstitial cell proliferation or on aberrant adreno-cortical tissue. Lack of response to a course of cortisone and a biopsy solved this problem.

Hormone analyses may be of much help. The estimation of total 17-ketosteroids in the urine is of limited aid in establishing the cause of macrogenitosomia praecox. Remarkably high figures are likely to be due to neoplasm either of the adrenal cortex or of the Leydig cell. According to Bishop *et al.* the urinary output

of 17-ketosteroids have been reported in only 11 of the 26 cases of Leydig cell tumour in children. The findings were of little significance in most of the cases as were the low figures in our case.

Congenital virilizing adreno-cortical hyperplasia is thought to be due to a faulty synthesis of Compound F. Butler & Marrian described in 1937 the presence of pregnanetriol in the urine of two women with the adrenogenital syndrome (2). This metabolite has later been demonstrated in greatly increased amounts in many patients with adreno-cortical hyperplasia. Pregnanetriol has also recently been demonstrated in urine from patients with adreno-cortical tumour. The conclusion seems justified from the literature that a normal figure for pregnanetriol (0-0.5

TABLE 1. 24-hr excretion of hormones and their metabolites in the urine of A. H.

	Nov. 59	9-11.1	12-14.1	25-27.1	6-9.2	22-25.2	24.5.60
Gonadotrophins M.U.	<48>6	<6	<6	<6	<2	Postop.	<9
17-KGS mg	11.1	6.1	4.5	7.7	1.1	4.9	5.4
17-KS mg	12.6	3.4	4.8	6.9		3.5	3.2
Pregnanetriol mg					0.3	0.8	
Pregnanediol				1.7	0.3		0.2
Fractionated 17-KS:							
Androsterone mg					1.1	1.0	1.3
Dehydroepiandrosterone mg					0.6	0.2	0.8
Aetiocholanolone mg					0.4	0.6	0.5

mg) in the urine for a patient with sexual precocity serves to exclude the possibility of the usual type of adreno-cortical hyperplasia (without hypertension). Except for the case of Savard *et al.* (4), the urinary pregnanetriol level has not been reported in a case of Leydig cell tumour despite this probably being the most important hormone analysis for the differentiation between Leydig cell tumour and adreno-cortical hyperplasia. Savard *et al.* demonstrated in their case increased urinary output of pregnanetriol. The tumour also had in other respects activities suggestive of adreno-cortical properties. A cortisone course was not attempted. The daily excretion of 0.3 mg pregnanetriol in our case was not compatible with a diagnosis of abberant adreno-cortical tissue causing the sexual precocity.

It is universally accepted that Leydig cells produce testosterone. The metabolites of this hormone excreted in the urine are mainly aetiocholanolone and androsterone, but the same two substances may also be metabolites of adreno-cortical hormones. Dehydroepiandrosterone, which in normal urine constitutes 10-30 per cent of the total 17-ketosteroids, is usually considered to be mainly a metabolite of adreno-

cortical androgens. The substance gives a strongly positive Allen Blue Test in most cases of adreno-cortical tumour and may be weakly positive in adreno-cortical hyperplasia. Cook *et al.* applied the micro-chromatographic fractionation procedure of Zygmuntowics to the urine from a case of Leydig cell tumour in a 5 year old boy (3). Staubitz *et al.* (5) and Bishop *et al.* (1) fractionated the urinary 17-ketosteroids from three cases of Leydig cell tumour. A preponderance of aetiocholanolone and androsterone together with a decreased amounts of dehydroepiandrosterone was present in these four cases. In the case reported by Savard *et al.* there was an increase of the alphaketosteroids (aetiocholanolone and androsterone) as well as dehydroepiandrosterone (and 11 oxygenated 17-ketosteroids). In our case we were not able to demonstrate an increased amount of the 17-alphaketosteroids but the method is probably of limited value for patients with a low total 17-ketosteroid output.

### Summary

A case of Leydig cell tumour in a 6½-year-old boy is presented. The need for more elaborate methods for the biochemi-

cal separation of Leydig cell tumour from aberrant adreno-cortical tissue is stressed. Normal urinary output of pregnanetriol and a lack of increase of the 17-beta-ketosteroids in the urine in a patient with a tumour in one of the testes serve to exclude the possibility of an adreno-cortical disorder.

### Addendum

Follow up: One year later, in February 1961, there still is no demonstrable alteration in the signs of precocity. The right testis is about the same size as in May 1960.

### References

1. BISHOP, P. M., VAN MEURS, D. P., WILLCOX, D. R. and ARNOLD, D.: Interstitial-cell tumour of the testis in a child. *Brit Med J*, 5168: 238-242, 1960.
2. BUTLER, G. C. and MARRIAN, G. F.: Chemical studies on adreno-genital syndrome; isolation of 3 (alfa) hydroxyetiocholane-17-one (isoandrosterone) and new triol from the urine of woman with adrenal hyperplasia. *J Biol Chem*, 124: 237-247, 1960.
3. COOK, C. D., GROSS, R. E., LANDING, B. H. and ZYGMUNTOWICS, A. S.: Interstitial cell tumour of the testis. Study of a 5-year old boy with precocious puberty. *J Clin Endocr*, 12: 725-739, 1952.
4. SAVARD, K., DORFMAN, R. I., BAGGETT, B., FIELDING, L. I., ENGEL, L. L., MCPHERSON, H. T., LISTER, L. M., JOHNSON, D. S., HAMBLIN, E. C. and ENGEL, F. L.: Clinical morphological and biochemical studies of a virilizing tumor of the testis. *J Clin Invest*, 39: 534-551, 1960.
5. STAUBITZ, W. J., OBERKIRCHER, O. J. and BLICK, M. S.: Precocious puberty in a case of bilateral interstitial cell tumor of testes. *J Urol*, 69: 562-570, April 1953.

Submitted Sept. 28, 1960

Accepted Nov. 1, 1960

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CASE REPORT

## Premature Twins with Esophageal Atresia and Tracheo-esophageal Fistula

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Esophageal atresia occurs approximately once in four thousand births. It rarely occurs in one member of twin siblings. Approximately 18 such infants are reported in the English literature (1). Six of these twins were "thought to be identical". To our knowledge, esophageal atresia occurring in both members of a pair of twins has not been previously reported (1, 2, 3, 4). This paper is the report of a pair of male twins, both of whom were born with esophageal atresia and tracheo-esophageal fistula, and both of whom survived surgical correction in spite of advanced prematurity. Because these twins are of the same sex, have a similar appearance, have identical anomalies and identical serologic phenotypes, they are believed to be genetically identical.

### Case Report

The twins were born on January 4, 1960, to a 33-year-old gravida III, para I, abortion I, Caucasian mother. Estimated duration of gestation was 34 weeks. Pregnancy was complicated by minimal vaginal bleeding during the third month. This responded to restriction of physical activity. During the third trimester there was an ab-

normally rapid increase in abdominal girth. At delivery this proved to be due to a marked excess of amniotic fluid. This is a common finding in mothers who give birth to infants with esophageal atresia because the amniotic fluid cannot reach the fetal intestinal tract for reabsorption (8, 9).

The mother and father each had a child or children by a previous marriage. None of these half-siblings had congenital defects. The twins were the first infants of this marital union. The mother and father have no congenital defects and none are known in relatives of either.

At birth infant A weighed 1672 g. Infant B weighed 1532 g. There was a single placenta with two umbilical cords originating adjacent to each other near the center of the placenta. There were two amniotic sacs. The placenta and membranes were not examined for separate or common chorions. Identical twins may have individual amniotic sacs but do not have individual chorionic envelopes (5).

Both twins were vigorous and no physical abnormalities were noted at birth. On the third day of life, feedings were attempted and were promptly regurgitated. X-rays revealed esophageal atresia and tracheo-esophageal fistula in each infant (Fig. 1). Pharyngeal suction was started immediately and surgical therapy planned. This was performed in a staged manner, as has been described in a previous communication (10). The mortality



Fig. 1. X-rays of twin A on the left and twin B on the right. Radiopaque dye has been placed in the esophageal pouch which ends blindly at the third dorsal vertebra. Notice gas in the abdominal viscera indicative of associated tracheo-esophageal fistula. The gas passes from the trachea through the lower esophageal segment into the stomach and intestine.

rate in premature infants with esophageal atresia has been unacceptably high when an anastomosis between the two esophageal segments has been performed as a primary definitive procedure. We now routinely treat premature infants by a staged regimen.

#### *Course of Infant A*

Infant A weighed 1442 g at age three days, when a Stamm gastrostomy was performed under local anesthesia (Figs. 2 and 3). Following this the infant continued to be vigorous, and twelve hours later under general endotracheal anesthesia the tracheo-esophageal fistula was doubly ligated. This procedure was performed extrapleurally through the bed of the fifth right rib (Fig. 4). The infant could then be fed by gastrostomy

without fear of regurgitation of feedings into the trachea (Fig. 5). A special nurse was in constant attendance to insure complete removal of pharyngeal secretions.

On January 3, 1960, fifteen days after ligation of the fistula there was evidence of formula leaking from the stomach through the esophagus and through the fistula into the trachea. The tracheo-esophageal fistula had reopened. Feedings were discontinued and tracheal suction performed to correct the resultant aspiration pneumonitis. The patient had gained 430 g fifteen days, now weighing 1872 g. The following day, after much of the aspiration pneumonitis had been corrected, the third surgical procedure was performed. Through a transpleural right thoracotomy the tracheo-esophageal fistula was divided and an end-to-end esophageal anastomosis performed (Fig. 6 and 7). Post-



operatively the infant developed a right upper lobe atelectasis which was corrected by endotracheal suction. The baby was fed by gastrostomy for two weeks at which time small oral feedings were started. These feedings were gradually increased until full feedings were being taken by mouth without difficulty. Weight gain was satisfactory and on February 24, 1960, 31 days after the esophageal repair and at age 51 days, the infant was discharged from the hospital weighing 2.76 kg. He has continued to gain weight and at age eleven months weighs 8 kg (Fig. 8).

### Course of Infant B

Infant B weighed 1332 g at age three days and was treated in a similar fashion. A Stamm gastrostomy was performed under local anesthesia as soon as the diagnosis had been made (Figs. 2 and 3). The patient tolerated this procedure well and remained vigorous. Twelve hours later the tracheo-esophageal fistula was doubly ligated by the extrapleural approach through the bed of the right fifth rib posteriorly (Fig. 4). A small opening was made in the pleura during the dissection, and postoperatively the infant developed a pneumothorax requiring tube thoracostomy. The following day gastrostomy feedings were started and a steady weight gain ensued. Since infant A

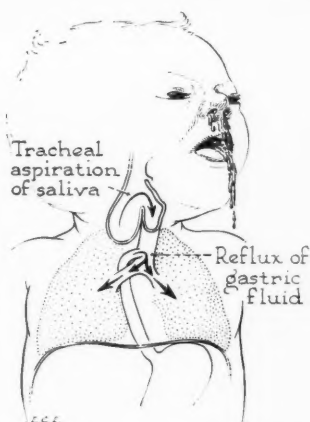


Fig. 2. The lungs are insulted from two sources. Saliva spills over from the upper esophageal pouch and gastric contents reflux into the trachea through the tracheo-esophageal fistula. (Used with permission from *Western J Surg*, WOOLLEY, M. M.: The premature infant with esophageal atresia.)

had had leakage from the fistula at age fourteen days, methylene blue was added to the formula of infant B so that a leak could be identified promptly, thus minimizing aspiration pneumonia. At age 26 days the methylene blue appeared in the pharyngeal suction, therefore, the gastrostomy feedings were stopped, and there was no evidence of

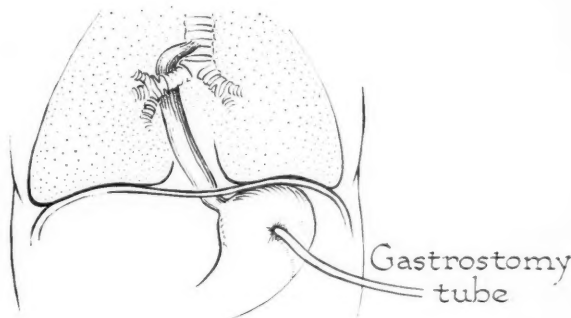


Fig. 3. A Stamm gastrostomy is performed under local anesthesia for subsequent gastrostomy feedings. (Used with permission from *Western J Surg*, *idem*.)

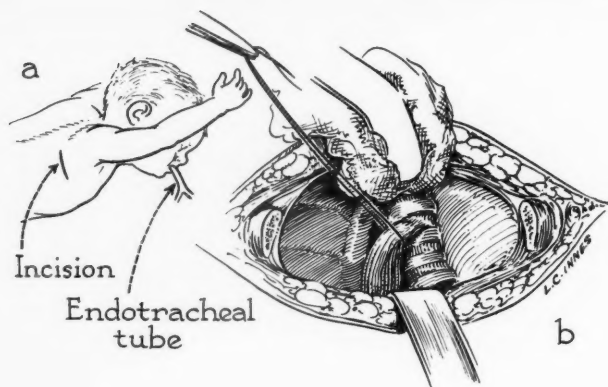


Fig. 4.

aspiration pneumonitis. The infant weighed 1895 g, a gain of 563 g in 22 days. The following day the tracheo-esophageal fistula was divided, and an end-to-end esophageal anastomosis was performed transpleurally (Fig. 6 and 7). Gastrostomy feedings were continued postoperatively, and oral feedings were started in small amounts and gradually

increased. Six weeks after the anastomosis the baby developed progressive difficulty with oral feedings, therefore a Dionasil (R) swallow was performed which showed a very tight esophageal stricture as well as recurrence of the tracheo-esophageal fistula (Fig. 9). On March 3, 1960, at age 69 days, the infant underwent the third thoracotomy. The fistula had recurred at the anastomotic site, and there was also a tight stricture of the esophagus with only a pinpoint lumen remaining. The fistula was reclosed, the stricture resected, and an end-to-end esophageal anastomosis performed. The infant again required endotracheal aspiration on several occasions postoperatively but progressed from gastrostomy feedings to oral feedings without difficulty. The weight gain was steady and the infant was discharged from the hospital on March 25, 1960, at age 81 days, weighing 3.06 kg. All feedings were being given by mouth, his gastrostomy tube having been removed. Weight gain was progressive, and there was no dysphagia until age five and one-half months at which time a single esophageal dilatation was required. This relieved both the dysphagia and wheezing which was due to pressure of the upper esophageal segment on the trachea. The infant's weight at eleven months was 8 kg (Fig. 8).

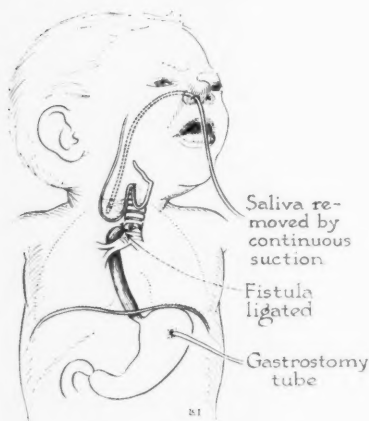


Fig. 5. Salivary secretions are removed from the upper pouch by a catheter. The infant is fed through the gastrostomy tube without fear of reflux of feedings into the trachea since the tracheo-esophageal fistula has been ligated.

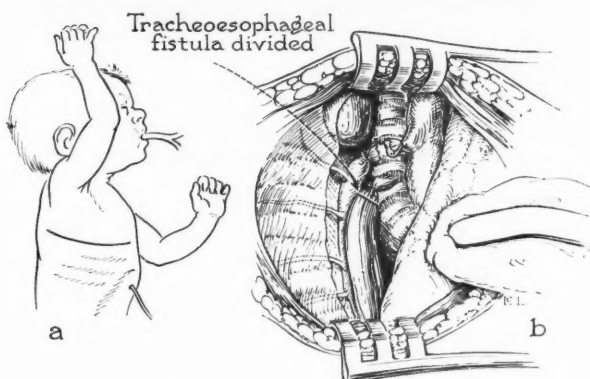


Fig. 6. (a) A long incision is made through the fourth intercostal space. The pleura is opened widely for good exposure. This is simpler than the extrapleural route. (b) The tracheo-esophageal fistula is divided, the previously placed ligature being visualized. (Used with permission of *Western J Surg, idem.*)

### Discussion

Haight (1957) reported a set of twins who were thought to be genetically identical, but only one of whom had esophageal atresia (1). The embryological reason for this is that monozygotic twins have their origin within two weeks of fertilization (6). The lung bud does not make its appearance until two weeks later at approximately four weeks after gestation. Still later, the trachea and esophagus develop by septation (6, 7). It becomes apparent then that, although twins may be genetically identical, that is, originating from one fertilized ovum, one of the twins may have esophageal atresia because of subsequent development in only one of the embryos. This is given as the reason for esophageal atresia not having occurred in both members of a set of identical twins. In other words, this may be embryologically a developmental defect rather than a genetically transmitted one. It would seem entirely possible, however, that this defect

could be transmitted in a genetic sense to each member of a pair of identical twins. This case report presents such a possibility. These twins are of the same sex (male), they have a similar appearance, they have an identical anomaly, and their blood types are identical (Table 1).

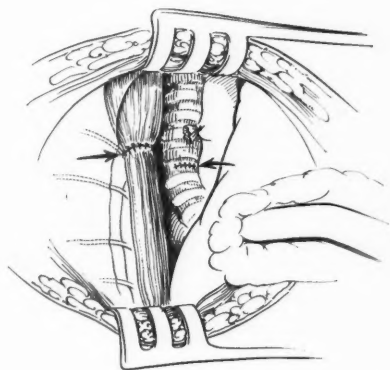


Fig. 7. The arrows indicate the esophageal anastomosis and the site of closure of the tracheo-esophageal fistula. (Used with permission of *Western J Surg, idem.*)



Fig. 8. This is a photograph of the twins at age eleven months. Twin A is on the right and twin B is on the left.

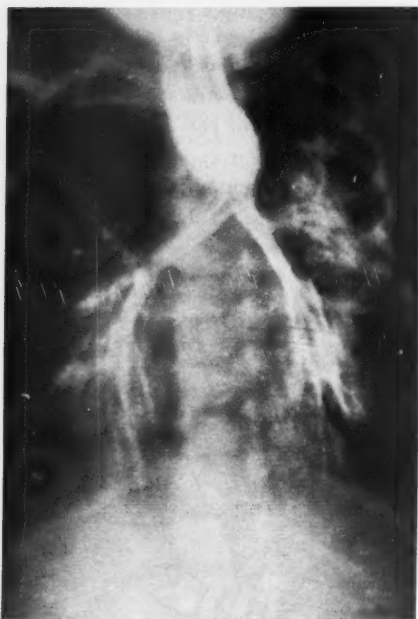


Fig. 9. X-ray of twin B. Dye was placed in the esophagus and immediately passed through a recurrent tracheo-esophageal fistula into the trachea and bronchi. Notice that no dye has passed through a very tight stricture into the distal esophagus.

TABLE 1. *Serological types of the mother and father and twins.*

	Mother	Father	Twin A	Twin B
Type	O	A	A	A
Rh	+	+	+	+
Kell	Neg.	Neg.	Neg.	Neg.
Duffy	Neg.	Pos.	Pos.	Pos.
MN	NN	MN	MN	MN
CDE	CDE/ce	cDE/ce	CDE/ce	CDE/ce

It will be noted that type A was contributed by the father, who may or may not have been homozygous for this characteristic. Both parents and infants are Kell negative, so that this blood factor contributes no information to the question of the twins' identity. They both inherited the Duffy-positive factor from their father, and they must both have inherited the M factor from their father, who was heterozygous for it, since their mother was N. The Rh types can be treated in terms of "most probable genotypes" from the test

results; these suggest that both the father and the mother were heterozygous at the Rh locus, and that both twins inherited the same allele from the heterozygous mother, and the same allele from the heterozygous father. These results permit a statistical deduction that the probability that these twins are fraternal is less than .05. This suggestion, that they are in fact identical, is derived from a statistical analysis of the serologic types alone, and does not take into account the supporting evidence from their appearance, sex, or any other more subjective findings.

In an analysis of deaths due to esophageal atresia it is found that the smaller the infant the less chance there is for his survival, if a primary esophageal anastomosis is performed. We have found that staged therapy for premature infants provides a much better chance for survival. Simple ligation of the tracheo-esophageal fistula is the quickest and easiest way of interrupting the fistula so that the infant can then be fed by gastrostomy. When he weighs more and is more mature, he is a better surgical risk, and a definitive correction can be performed at that time.

Recurrence of the tracheo-esophageal fistula, which occurred in twin B, is an uncommon complication occurring in approximately 1% of these infants. This

complication is probably due to an infection at the anastomotic site which drains simultaneously into the trachea and esophagus, thus reforming the fistula. There was no evidence of infection at the anastomotic site in this infant, but it could have cleared by the time surgery was performed.

### Summary

To our knowledge, this is the first reported case of esophageal atresia occurring in both members of a pair of twins. These twins appear to be genetically identical because of their general appearance, sex, identical anomaly, and identical serologic genotypes. Genetic predisposition for esophageal atresia in twins would seem to be possible from this study. Heretofore, it has been thought that esophageal atresia in one of identical twins has been a developmental defect rather than a genetically transmitted one. Staged therapy for the premature infant with esophageal atresia increases the chance of survival of these infants.

### Acknowledgements

The statistical analysis of the serological types was performed by Ray Owen, Ph. D., Professor of Biology, California Institute of Technology, Pasadena, California.

### References

1. HAIGHT, C.: Some observations on esophageal atresia and tracheo-esophageal fistula of congenital origin. *J Thor Surg*, 34: 141-172, 1957.
2. — Personal communication.
3. POTTS, W.: Personal communication.
4. GROSS, R. E.: *Surgery of Infancy and Childhood*. Philadelphia and London, W. B. Saunders & Co., 1953.
5. GROSS, R. E. and WOOLLEY, M. M.: Esophageal atresia and tracheo-esophageal fistula: a analysis of 100 consecutive cases. (Unpublished.)
6. AREY, L. B.: *Developmental Anatomy. A Text Book and Laboratory Manual of Embryology*. Philadelphia and London, W. B. Saunders & Co., 1946.
7. NELSON, O. E.: *Comparative Embryology*

- of the Vertebrates. New York, The Blakiston Co., Inc., 1953.
8. BREMER, J. L.: Congenital Anomalies of the Viscera. Boston, Harvard Press, 1957.
9. LLOYD, J. R. and CLATWORTHY, H. W.: Hydramnios as an aid to the early diagnosis of congenital obstruction of the alimentary tract: a study of the maternal and fetal factors. *Pediatrics*, 21: 6: 903-909, 1958.
10. SCOTT, J. S. and WILSON, J. K.: Hydramnios as an early sign of esophageal atresia. *Lancet*, 569-572, September 21, 1957.
11. WOOLLEY, M. M.: The premature infant with esophageal atresia. *Western J Surg*, In print.

Submitted Sept. 21, 1960

Accepted Oct. 20, 1960

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## REVIEW ARTICLE

### Peptic Ulcer in Children

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Peptic ulcer is generally considered to be an unusual disease in childhood. Isolated case reports are to be found in the literature, but accounts of any larger series are rare. In 1949 Karlström reported 30 cases diagnosed at 25 paediatric clinics in Sweden during a period of 5 years (3). His work has been of value in attracting attention to the incidence of peptic ulcer in children. His study did not, however, include a detailed account of the clinical features of the condition. More recently, Ramos *et al.*, presented a series of 32 cases which they analysed from different points of view (4). Several of these cases, however, had been first diagnosed as adults and had been included in the material because the typical history dated from childhood.

During recent years a striking number of children have been treated for peptic ulcer at the Gothenburg Children's Hospital. To obtain some idea of the incidence and clinical character of the disease, we have studied all cases of peptic ulcer treated here since 1945. This hospital has the advantage of being the only children's hospital catering for a well-defined district. A special study has been made of those

cases which have been under observation for more than 5 years. In order to enlighten certain factors of aetiology, a number of the most recent cases have been subjected to special psychiatric-psychological examination.

#### *Incidence, Age and Sex Distribution, Type of Ulcer.*

Since 1945, a total of 36 patients with the verified diagnosis "peptic ulcer" have been admitted to this hospital on one or more occasions. The diagnosis was confirmed by X-ray in 35 patients, and at operation in the remaining one. The number of new cases from the hospital's admission district, for the last three 5-year periods, were 4, 10 and 17 respectively, which demonstrates a definite increase in incidence (Fig. 1). This increase cannot be explained by an increase in local population, nor, probably, by improved diagnosis. It is our impression that a true increase has taken place in the incidence of peptic ulcer in children. Among the grounds for this belief is the fact that most patients displayed such typical signs and symptoms that diagnosis was virtually inevitable.



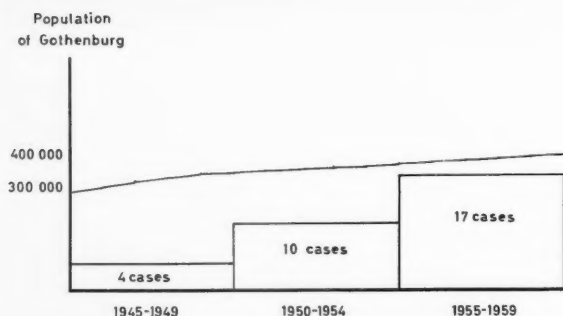


Fig. 1. The number of new cases of peptic ulcer discovered at The Gothenburg Children's Hospital during the three last 5-year periods related to the population served by this hospital during the same periods of time.

All patients except one were 9 years or older at the time of first admission (Fig. 2). In some it was possible to obtain a typical history dating from the age of 6-7 years. The disease appears to be exceptional in the pre-school age group.

Our series includes only one such case. This was a girl, aged 23 months at the time of admission, in whom ulcer was discovered at operation undertaken because of a 2 week history of increasing vomiting of the obstructive type. Laparotomy revealed a little radiating ulceration at the junction between the gastric and duodenal mucosa. Considerable muscle hypertrophy was noted which caused severe stenosis of the pylorus. According to the operation notes, this hypertrophy was not similar to that of "congenital" hypertrophic pyloric stenosis.

Our material consists of 27 boys and 9 girls. Thirty-one children had duodenal ulcer, and 5 had gastric ulcer.

### Symptoms

Table 1 illustrates the incidence of the different symptoms, regardless of the stage in the disease at which each symptom appeared. Abdominal pain was present in all but one child. A striking number of

TABLE 1. Incidence of the different symptoms, regardless of when they appeared, in the course of the disease.

<i>Pain</i>	35
Delayed pains, relief after food	26
Nocturnal pains	15
Pain related to meals	11
Atypical pains	5
<i>Tenderness on palpation</i>	16
<i>Periodicity</i>	19
<i>Heartburn, regurgitation</i>	19
<i>Vomiting</i>	17
Haematemesis	4
Obstructive vomiting	2
Vomiting in uncomplicated ulcer cases	11
<i>Haemorrhage</i>	8
Macroscopic	5
Occult	3
<i>Anaemia</i>	5
<i>Anorexia</i>	9
<i>Loss of weight</i>	7
<i>Fatigue</i>	7

patients complained of typical delayed pains, or admitted relief of pain after food. In 18 patients this typical ulcer history led to the diagnosis. Nocturnal pains were also quite a common feature, and were of diagnostic significance in 4 cases where pains during the day were

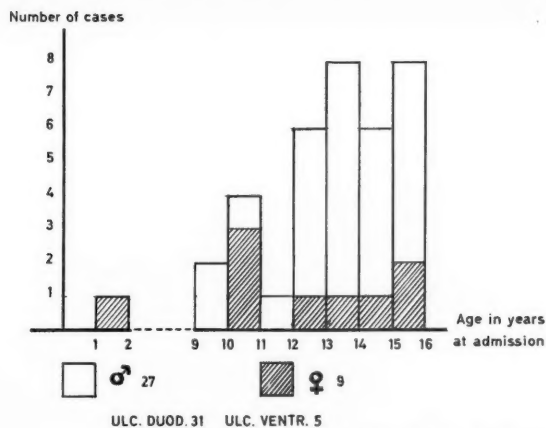


Fig. 2. Age, sex distribution of children with duodenal or gastric (ventr.) ulcer.

absent or atypical. Pain directly related to meals could usually be attributed to a certain type of food. In only 2 children was this a dominant feature prompting X-ray examination of the stomach. Five patients had atypical pains throughout the course of their disease.

Heartburn and/or regurgitation were noted in 19 patients. Histamine test meal was performed in 11 cases; there was hypersecretion in 3, normal findings in 6, while 1 case showed hyposecretion and 1 "gastric values".

In previous descriptions of peptic ulcer in children, vomiting and haemorrhage have been given as leading symptoms. In our series only 5 patients with uncomplicated peptic ulcer had repeated vomiting as an important symptom. In these cases vomiting was related to severe abdominal pains, and brought about a relief of pain. Obstructive vomiting was seen in 2 patients, in one of whom it was the only symptom. Haematemesis occurred in 4 of the 5 patients with macroscopic bleeding

and was the reason for admission to the hospital. Occult blood was discovered in 3 children. Five patients were anaemic, but in 2 no active bleeding could be demonstrated. There was no instance of perforation during the period of study.

Table 2 illustrates the symptoms which were of diagnostic significance in the particular cases. The duration of symptoms up to the time of diagnosis was usually

TABLE 2. Predominating and diagnostic symptom at the time of (the first) admission, together with the duration of the symptoms prior to admission.

	No. of cases	Duration in years			
		Under 1	1-2	2-3	Over 3
Typical ulcer history	18	10	2	2	4
Nocturnal pains	4		1	1	2
Pain at mealtimes	2	2			
Atypical pains	5	3	1		1
Haemorrhage	5	2	2		1
Miscellaneous	2	1			1
Total	36	18	6	3	9

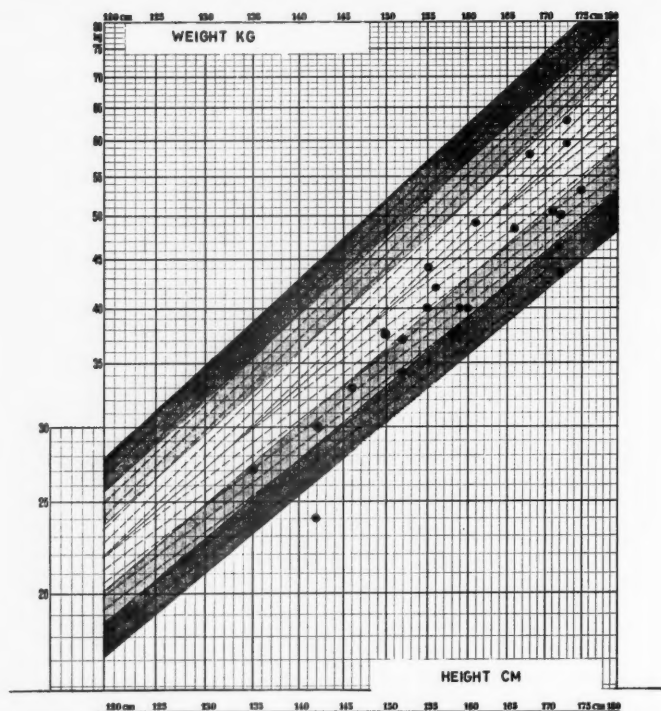


Fig. 3. Height and weight of the boys on (the first) admission to hospital.

clear from the case history and varied from one month to several years. Seventeen patients had a history of less than 6 months. However, some had had symptoms for several years and could not state the duration with any precision.

It is striking that many patients gave a quite typical history of the disease. It does not appear to have been difficult to differentiate children with ulcer from those with functional conditions causing abdominal pains. The latter does not seem to be particularly common among children with ulcer. In a few children, vague types of abdominal pain preceded typical ulcer symptoms sometimes for a long period.

### Treatment and Prognosis

The great majority of patients quickly became symptom free on the usual regimen of rest, diet and medical treatment. The time taken to achieve healing, as confirmed radiologically, was more variable. In some this has not been done.

Three patients were operated upon before they reached the age of 16 because of failure of medical treatment, or repeated recurrences. Eleven of 28 patients had at least one recurrence before 16 years of age.

Follow-up was performed in 14 children in whom the observation period from the first hospital admission was more than 5 years (Table 3). Only 2 of these were free

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TABLE 3. *Follow-up study of 14 cases which have been under observation for more than 5 years.*

One or more relapses confirmed by X-ray	9
Treated by operation	5
Conservative treatment only	4
Typical ulcer symptoms, not necessitating admission or sick leave	3
Symptom-free	2

from symptoms. The prognosis can therefore be said to be unfavourable.

### Aspects of Aetiology

From an aetiological point of view ulcers fall into 2 categories. The first type appears secondarily to some other severe illness. Our series contains no certain case of this kind. The other kind of ulcer is a primary, chronic disease considered to be psychosomatic in origin (6). This latter type may presumably occur in susceptible individuals under certain external circumstances.

In 25 of our patients (circa 70%) there was a family history of peptic ulcer, usually in one of the parents. This figure is higher than in a series of adults and would suggest that constitutional factors are important in the causation of the lesion (4). We have studied the incidence of certain constitutional features which are considered to be associated with a predisposition to ulcer.

The sex distribution has already been discussed. In our series boys were definitely in the majority. Fig. 3 illustrates the boys' weight and height on admission to hospital. Most of them fall in the area of low body weight in relation to height. This could possibly be accepted as leptos-

som habitus, since loss of weight was an unusual and less prominent symptom.

Blood group O was found in 13 of the 21 grouped patients, i.e. in 62% as against an expected 38% (1).

Terman-Merrill testing was carried out in 16 patients and the mean I.Q. was found to be 110, indicating that children of above-average intelligence are over-represented among peptic ulcer patients. A psychiatric opinion was given in 14 cases. Of 11 children, whose psychical constitution could be determined, 6 were considered psycho-asthenic.

The psychiatric examination also included an evaluation of environment. In 6 cases stress factors were discovered (broken homes in 2 cases, threats of divorce, long-drawn family dispute, alcoholic step-father, and psychic strain at school and work). Five patients were considered to have good home environment. The remaining 3 formed an intermediate group.

As a rule the patients feeding habits were gone into and irregular or unsatisfactory meals were admitted in only 2 cases. Feeding difficulties in infancy, organic abdominal disease or other somatic disturbances did not appear to be common. No patient had had hypertrophic pyloric stenosis in infancy.

Projective test methods in children may indicate the existence of significant emotional disturbances. Rorschach testing was performed in 14 cases. The results did not suggest that neuroses or emotional disturbances were common and prominent among children with ulcer (6). All patients tested gave both a high number of answers and whole answers, which is characteristic of high intelligence. But even if

the I.Q. is taken into consideration, the number of answers and whole answers, is unusually high. This is usually interpreted as an ambitious attitude. These results were in agreement with the clinical impressions of the patients.

In spite of the limited material, it would appear that constitutional factors are important in the causation of peptic ulcer in children. Factors of environment appear to be less obvious. This could be taken as signifying that patients affected with peptic ulcer in childhood comprise a selection of children with particularly strong predisposition to the disease.

### Summary

A study has been made of 36 children with the established diagnosis peptic ulcer. All patients, with one exception, were children of school age. The following aspects of the disease have come to light.

1. The incidence of peptic ulcer among older children appears to be increasing.
2. These patients often present a strikingly typical history. Delayed pains and relief of pain by food were noted in 26 patients. Other typical ulcer symptoms occur commonly.

3. Macroscopic bleeding was discovered in 5 cases, obstructive vomiting in 2. There was no case of perforated ulcer.

4. Recurrence was common. Of 14 children observed for a minimum period of 5 years, only 2 had been symptom-free.

5. Constitutional factors are important in the causation of peptic ulcer in childhood. A high proportion of patients have a family history of the disease. Boys are affected more than girls. Patients of blood group O are present in abnormally high proportion. Psycho-asthenic constitution is common among these patients.

Environmental factors appear to be less obvious. Neuroses or emotional disturbances were not found to any great extent.

### Acknowledgement

We are greatly indebted to Mr S. Dworetzky, chief psychologist at the Dept. of Child Psychiatry, who has performed the psychological examinations.

### References

1. BUCKWALTER, J. A., WOHLWEND, E. B., COLTER, D. C., TIDRICK, R. T. and KNOWLER, L. A.: Peptic ulceration and ABO blood groups. *JAMA*, 162: 1215, 1956.
2. CHAPMAN, A. H., LOEB, D. G. and YOUNG, J. B.: A psychosomatic study of five children with duodenal ulcer. *J Pediatr*, 48: 248, 1956.
3. KARLSTRÖM, F.: Ulcus-Krankheiten beim Kinde mit besonderer Berücksichtigung der Häufigkeit. *Helv Paediat Acta*, 4: 455, 1949.
4. RAMOS, A. R., KIRSNER, J. B. and PALMER, W. L.: Peptic ulcer in children. *Amer J Dis Child*, 99: 135, 1960.
5. STENBÄCK, A.: Cardial ångestneuros och ulcus duodeni. *Nordiskt Psykiatriskt Medlemsblad*, 11: 89, 1957.
6. WRETMARK, G.: The peptic ulcer individual. *Acta Psychiat Scand Suppl.* 84, 1953.

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Submitted Dec. 15, 1960

Accepted Dec. 19, 1960

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## A Follow-Up Study of Hyperbilirubinaemia in Full-term Infants without Iso-immunisation

by J. BJURE, G. LIDÉN, T. REINAND and A. VESTBY

The harmful effects of hyperbilirubinaemia in haemolytic disease of the newborn are well documented, and the indications for treatment generally accepted. Exchange transfusion has been recommended when the serum bilirubin level exceeds 18 mg % (11) or 20 mg % (3, 5, 7). There is uncertainty, however, about the deleterious effect of hyperbilirubinaemia in babies with severe neonatal jaundice without evidence of haemolytic disease. Practice differs as regards exchange transfusions in these cases. The purpose of this investigation is to elucidate this problem by a follow-up study of deeply jaundiced full-term newborns without evidence of haemolytic disease. The findings in infants who were treated by exchange transfusion are compared with those in untreated patients.

### Material and Methods

The material comprises 113 full-term infants, born at the two maternity hospitals in Gothenburg, during the years 1956 and 1957. In all infants the serum bilirubin level rose to 18 mg % or more. The infants were divided into three groups. Group I (uncomplicated

cases): comprise babies in whom the pregnancy, delivery and neonatal period were completely normal with the exception of hyperbilirubinaemia; Group II (slightly complicated cases) include those in whom there were slight complicating factors in pregnancy or delivery such as simple proteinuria of the mother, certain types of medication administered to the mother without obvious effect to the infant, or the umbilical cord loosely around the child's neck, which probably did not affect the infant; Group III (complicated cases) consists of cases with complications which might be deleterious to the child, including asphyxia, abnormal presentations, toxæmia, or surgical intervention. Some infants in all three groups were treated by exchange transfusion, though more in Group III than in the others.

The control series comprises 51 full-term infants born during the same period following normal pregnancies and uncomplicated deliveries and without marked icterus or other complications in the new-born period. The whole material is listed in Table 1.

A direct Coombs test was performed on all jaundiced infants, and was negative. In 43 an indirect Coombs test was also done; this, too, was invariably negative. Bilirubin determinations were performed on capillary finger or heel blood by the method of Jendrassik-Grof (8). In order to determine the incidence of severe hyperbilirubin-

This investigation was made possible by a grant from "Majblomman" Fund.

TABLE 1. *Total Number of Children Examined in Different Groups.*

Uncompl. cases		Slightly compl. cases		Compl. cases		Controls
No exchange transf.	Exchange transf.	No exchange transf.	Exchange transf.	No exchange transf.	Exchange transf.	
23	4	29	10	29	18	
	27		39		47	
		66		47		51

aemia during the neonatal period serum bilirubin determinations have been performed during a one year period in all infants with jaundice. Of 3,597 full-term infants born at one of the maternity hospitals during 1957 a total of 47 or 1.3% had a bilirubin of 20 mg per 100 ml or more, which is roughly in accordance with a recently published study (9).

The follow-up study, which has included physical and neurological examination, as

well as Merrill-Palmer testing (15), was performed at an age of 2-3 years. On the slightest deviation from normal the child was subjected to psychiatric examination; this was done in 25 infants. The following features have been taken as symptoms of early brain damage: 1. Hyperactivity, restlessness and inability to concentrate; 2. Stereotypic behavior; 3. Impairment of voluntary movement (clumsiness, fumbling, late motor development); 4. Mental retardation. Since none of these features is pathognomonic of early brain lesion, the diagnosis has been made only where it was impossible to explain the symptoms by other causes, such as environmental factors. Play audiometry was also performed in 125 children, 86 with jaundice and 39 controls.

### Results

No signs of kernicterus were demonstrable during the newborn period. Fig. 1 shows the age when maximal bilirubin levels were found in the different cases, the result being in agreement with what has been previously reported (12). Exchange transfusions were performed on the day of the highest bilirubin level.

On general physical examination no relevant abnormalities were noted. The results of Merrill-Palmer test are shown in Tables 2 and 3. No difference was found between the groups regarding I.Q. distribution (Table 2). When the material is classified according to the level of hyperbilirubinaemia there is still an apparently normal I.Q. distribution in all groups (Table 3). It can be seen that the distribution of patients with respect to bilirubin-concentration is also approximately the same in both groups.

Table 4 shows the number of infants examined by means of play audiometry. No deafness of the central type was revealed.

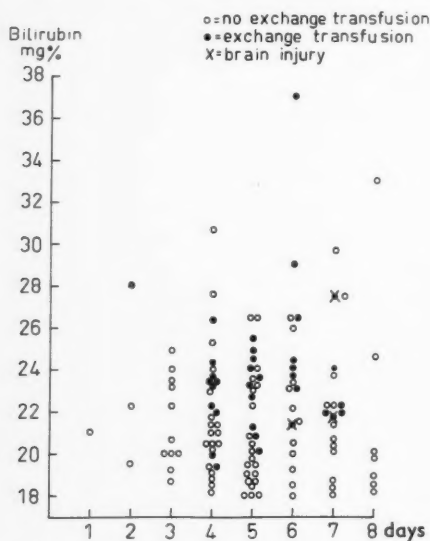


Fig. 1. Maximum bilirubin level in each infant.



TABLE 2. *I.Q. Distribution within Different Groups.*

IQ	Uncompl. cases		Slightly compl. cases		Compl. cases		Controls
	No exchange transf.	Exchange transf.	No exchange transf.	Exchange transf.	No exchange transf.	Exchange transf.	
150	—	—	—	—	—	—	—
140	—	—	1	—	—	1	—
130	3	1	—	—	1	—	5
120	5	—	5	—	1	2	5
110	2	1	6	2	9 <sup>1</sup>	5	5
100	7	2	9	4	8	3	21
90	4	—	5	3	8	7 <sup>3</sup>	10
80	2	—	1	1	1	—	3
70	—	—	2	—	—	—	2
60	—	—	—	—	—	—	—
50	—	—	—	—	1 <sup>2</sup>	—	—
Total	23	4	29	10	29	18	51

1, 2, 3 = Cases with brain injury.

TABLE 3. *I.Q. Distribution in Relation to the Level of Hyperbilirubinaemia.*

IQ	Uncompl. cases + slightly compl. cases			Compl. cases			
	Bilirubin in mg%						
	18-19.9	20-24.9	25 +	18-19.9	20-24.9	25 +	Controls
150	—	—	—	—	—	—	—
140	—	1	—	—	1	—	—
130	1	3	—	—	—	1	5
120	2	6	2	2	2	—	5
110	3	7	—	5	7 <sup>1</sup>	2	5
100	5	15	3	1	8	2	21
90	5	6	1	3	7	4 <sup>3</sup>	10
80	1	2	1	1	—	—	3
70	—	2	—	—	—	—	2
60	—	—	—	—	—	—	—
50	—	—	—	—	1 <sup>2</sup>	—	—
Total	17	42	7	12	26	9	51

1, 2, 3 = cases with brain injury.

The incidence of a suspected brain lesion in the 25 children examined by the child psychiatrist is shown in Table 5. The 3 cases in whom a brain lesion was diagnosed belonged to Group III (complicated case). One of them had been treated by

exchange transfusion. Symptoms suggestive of brain lesion were recognised in 9 children, but in 6 of these the symptoms could be alternatively explained. In the remaining 3 cases the diagnosis of a brain lesion could be made with confidence.

TABLE 4. *Cases Examined with Play Audiometry.*

Uncompl. cases		Slightly compl. cases		Compl. cases		Controls
No exchange transf.	Exchange transf.	No exchange transf.	Exchange transf.	No exchange transf.	Exchange transf.	
15 (23)	4 (4)	21 (29)	10 (10)	21 (29)	15 (18)	39 (51)

Total number of children within brackets.

TABLE 5. *Psychiatrically Examined Children.*

	Uncompl. cases		Slightly compl. cases		Compl. cases		Controls	Total
	No exchange transf.	Exchange transf.	No exchange transf.	Exchange transf.	No exchange transf.	Exchange transf.		
No. of psychiatric exam. children	3	1	5	2	10	2	2	25
Symptoms:								
Hyperactivity, Restlessness, Lack of concentration.	1	—	—	—	2 <sup>1</sup>	1 <sup>3</sup>	—	4
Stereotypic behaviour.	—	—	1	—	2	—	—	3
Motor disorders	—	—	1	—	1 <sup>2</sup>	—	—	2
Mental retardation	1	—	1	—	1 <sup>2</sup>	—	—	3

1, 2, 3 = cases with brain injury.

*Case 1.* Boy, born 29.8.56, birth weight 3.720 g, second child of a 33 year old woman, whose first pregnancy was in 1940. Except for vomiting during the first months the pregnancy was normal. Delivery occurred after a 2 1/2 hour labour with left occipito-anterior presentation. No signs of asphyxia in utero were present but slight extrauterine asphyxia occurred accompanied by marked petechial haemorrhages on the face. Blood groups: mother O Rh +, child O Rh +; Coombs direct test negative. Icterus was noted with a maximum bilirubin value of 21.8 mg % on the 7th day. The child was able to sit at 6 months, walked at 1 year, and talked at 21 months. Suspected ECHO infection at 1 year, and convulsions in association with fever at 18 months. Somatic examination 22.1.59 showed nothing abnormal. I.Q. 22.1.59 was found to be 110.

Psychiatrist's opinion on examination 9.9.59: "Marked hyperactivity, restlessness, lack of concentration and lack of inhibition which cannot be explained by deficiencies of environment. Brain lesion syndrome without intelligence defect."

Comment: Quick labour with considerable variations in pressure, congestive haemorrhage.

*Case 2.* Boy, born 3.7.56, birth weight 3.350 g. First child of a 34 year old mother who had aborted in the 3rd month of a previous pregnancy in 1955. Proteinuria was noted towards the end of the pregnancy. Low forceps delivery occurred following 22 hours labour with the head presenting in left occipito-anterior position. Indication: green liquor and weak pains. The umbilical cord was wound tightly around the neck, on which an impression could be seen. Slight extra-uterine

asphyxia occurred. Blood groups: mother A Rh +, child A Rh +, direct Coombs test negative. Icterus appeared on the 2nd day, and the maximum serum bilirubin level, 21.3 mg %, was recorded on the 6th day. Development was retarded, unable to sit at 1 year, starting to walk at 2 years. He had repeated respiratory infections during the first year of life. Physical examination 26.2.59, revealed nothing abnormal except the retarded development. Testing 20.5.59, showed an I.Q. of 55. Psychiatric opinion 30.9.59: "Difficult to get into contact with, clumsy movements, patient severely retarded, but not restless or lacking concentration. Unfavourable environment. Brain lesion syndrome with mental defect, together with environmental injury".

Comment: Rather protracted delivery with signs of intra- and extra-uterine asphyxia.

Case 3. Boy, born 2.7.57, birth weight 3.080 g, first child of 33 year old mother. Delivery by breech presentation following a 4 hour labour. Blood groups: mother O Rh +, child O Rh +. Direct and indirect Coombs tests negative. Icterus developed on the 2nd day with a maximum bilirubin level of 27.5 mg % on the 7th day. Exchange transfusion was performed. The child could sit without support at the age of 7 months; he walked at 11 months, and formed a few sentences at 26 months. Physical examination 2.9.59, revealed no definite abnormality. Testing 25.9.59, showed an I.Q. 96. Psychiatric examination 24.11.59: Severe restlessness and lack of concentration, contact only superficial and of short duration. Favourable environment. Brain lesion without intelligence defect.

Comment: Quick labour, breech delivery.

### Discussion

In a recent publication (12) Mores *et al* concluded that hyperbilirubinaemia without iso-immunisation does not involve risk of brain damage to healthy, full-term

infants, though details of the time and nature of follow-up examinations were not included. In the same report, a follow-up survey of premature infants with hyperbilirubinaemia in the absence of iso-immunisation was given. A much lower incidence of kernicterus was found than has been noted by other workers (1, 3, 4). Exchange transfusion was not performed in either the full-term nor premature infants reported by Mores *et al* and on the basis of their results they suggested that exchange transfusion was unnecessary in hyperbilirubinaemia, unless iso-immunisation was present.

Experiments have shown that an important factor in the causative mechanism of brain injury due to hyperbilirubinaemia is the functional maturity of the blood-brain barrier (6). Prematurity and asphyxial or traumatic injury at the time of labour might impair the blood-brain barrier, and thus increase the risk of a brain lesion due to hyperbilirubinaemia. Overdosage of such vitamin K analogues as Synkavit is thought to be a possible cause of hyperbilirubinaemia due to increased haemolysis with risk of brain damage (2, 10). In our series the total dose of vitamin K (menadiolsodiumsulphate) has never been more than 5 mg. None of our patients has been treated with gantrisin (14). In all 3 of our patients who at follow-up were found to have cerebral damage there were circumstances other than hyperbilirubinaemia to account for injury at the time of birth. This is not to say with certainty that hyperbilirubinaemia might not have had a contributing factor to the brain lesion. We have not found any evidence that in the absence of haemolytic disease hyperbilirubinaemia *alone* in full-

term, vigorous infants gives rise to cerebral lesions, irrespective of whether or not exchange transfusion is performed. It would appear that the custom of performing exchange transfusion on these infants could be largely dispensed with, a change which would be of considerable practical advantage since hyperbilirubinaemia has such a high incidence. It is however, not unlikely, that exchange transfusion is worth while in those cases where hyperbilirubinaemia is associated with circumstances favourable to impairment of the blood-brain barrier, or to the liberation of bilirubin from an albumin-bilirubin complex, as for instance after gantrisin medication or with a low serum albumin concentration (13).

The incidence of infants with hyperbilirubinaemia is much higher than corresponding figures from England (16). This may possibly be explained through the higher dosage of prophylactically given vitamin K. This problem is under investigation.

## Summary

One hundred and thirteen full-term infants with bilirubin values of 18 mg% or more during the neonatal period, have been followed-up at the age of 2-3 years, and compared with 51 full-term, non-icteric infants, born during the same period. Exchange transfusion was carried out in 30 of the children with jaundice. No cases of kernicterus could be demonstrated. Play audiometry was carried out in 125 children (86 with jaundice, 39 controls). No deafness of central type was revealed. At the paediatric follow-up examination no relevant abnormalities were found. Merrill-Palmer testing produced evidence of a similar I.Q. distribution among the icteric as among the non-icteric children. A cerebral lesion was diagnosed in 3 cases, but in all these another aetiology (disturbance at the time of birth) occurred in addition to hyperbilirubinaemia. Hyperbilirubinaemia alone did not give rise to any cerebral lesion.

## References

1. BAKKER, J. C. W.: Über den Icterus gravis und Kernicterus bei Frühgeburten ohne nachweislichen ABO- und Rhesus-Blutgruppenantagonismus. *Acta Paediat (Upps)*, 48: 529, 1954.
2. BOUND, J. P. and TELFER, T. P.: Effect of vitamin K dosage on plasma bilirubin levels in premature infants. *Lancet* I: 720, 1956.
3. BROWN, A. K. and ZUELZER, W. W.: Studies in hyperbilirubinaemia. I. Hyperbilirubinaemia of the newborn unrelated to isoimmunisation. *Amer J Dis Child*, 93: 263, 1957.
4. CROSSE, MARY V., WALLIS, PATRICIA G. and WALSH, ANNA M.: Replacement transfusion as a means of preventing kernicterus of prematurity. *Arch Dis Child*, 33: 403, 1958.
5. DIAMOND, L. K., ALLEN, F. H. JR., VANN, D. D. and POWERS, J. R.: Erythroblastosis foetalis; Round table discussion. *Pediatrics* 10: 337, 1954.
6. ERNSTER, L., HERLIN, L. and ZETTERSTRÖM, R.: Experimental studies on the pathogenesis of kernicterus. *Pediatrics*, 20: 647, 1957.
7. HSIA, D. Y.-Y., ALLEN, F. H., JR., GELIS, S. S. and DIAMOND, L. K.: Erythroblastosis foetalis. VIII. Studies of serum bilirubin in relation to kernicterus. *New Engl J Med*, 247: 668, 1952.
8. JENDRASSIK, L. and GRÓF, P.: Vereinfachte photometrische Methode zur Bestimmung des Blutbilirubins. *Biochem. Z* 297: 81, 1938.
9. KILLANDER, A., MÜLLER-EBERHARD, U. and SÖLIN, S.: Indications for exchange transfusion in newborn infants with hyperbilirubinaemia not due to immunization. *Acta Paediat (Upps)*, 49: 377, 1960.
10. MEYER, T. C. and ANGUS, J.: The effect of

large doses of "Synkavit" in the newborn. *Arch Dis Child*, 31: 75, 1956.

from albumin and its clinical implications. *J Pediat*, 55: 268, 1959.

11. MOLLISON, P. L. and CUTBUSH, MARIE: A method of measuring the severity of a series of cases of haemolytic disease of the newborn. *Blood*, 6: 777, 1951.

14. SILVERMAN, W. A., ANDERSEN, D. H., BLANC, W. A. and CROZIER, D. N.: A difference in mortality rate and incidence of kernicterus among premature infants allotted to two prophylactic antibacterial regimens. *Pediatrics*, 18: 614, 1956.

12. MORES, A., FARGAŠOVÁ, I. and MINÁŘIKOVÁ, E.: The relation of hyperbilirubinaemia in newborns without isoimmunization to kernicterus. *Acta Paediat (Upps)*, 43: 529, 1954.

15. STUTSMAN, R.: Guide for Administering the Merrill-Palmer Scale of Mental Tests. World Book Company. New York 1948.

13. ODELL, G. B.: The dissociation of bilirubin

16. ZETTERSTRÖM, R.: Personal communication.

Submitted Dec. 12, 1960

Accepted Dec. 19, 1960

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## Gamma Globulin against Rubella in Pregnancy

### *I. Prevention of Maternal Rubella by Gamma Globulin and Convalescent Gamma Globulin: a Follow-Up Study*

by ROLF LUNDSTRÖM, CLAES THORÉN and BERNT BLOMQUIST

#### Introduction

Gregg's [9] discovery in 1941 of maternal rubella as a cause of congenital abnormalities has been clearly confirmed in numerous studies on the subject. Different methods of collecting data have given varying results with respect to the incidence of congenital anomalies after maternal rubella in early pregnancy. Siegel & Greenberg [26] presented some evidence that the incidence may differ depending on occurrence of rubella during epidemic or non-epidemic years. The risk is high enough [5, 17, 20, 24] to make any attempt to prevent the disease in early pregnancy well worth while [20, 24].

Active immunization programmes with deliberate exposure of young girls have been attempted [1, 11]. The practical difficulties of inducing immunity in large populations along these lines limit the value of such trials. As long as the rubella virus has not been isolated and no effective vaccine has been produced, there will always be a certain number of susceptible fertile women, who should avoid contact with cases of rubella in early pregnancy. This is not, however, always possible. Consequently, when such a

woman has been exposed to rubella after recent conception, the only conceivable method of protecting her is by passive immunization, e.g. with convalescent gamma globulin or ordinary pooled gamma globulin.

#### Earlier Investigations

In the past, several attempts to protect pregnant women and other susceptible subjects by passive immunization have been made. Table 1 summarizes a number of studies in this field, mostly showing favourable results, but with attack rates in the treated subjects ranging from 0 to 53%. Even though passive immunization seems to have had a protective effect against the development of manifest signs of maternal rubella in many instances, this is not necessarily equivalent to success in the prevention of defects, since inapparent disease may cause damage to the foetus [25]. Rubella without a rash has also been described [15].

Little is known of the results of pregnancies in which passive immunization against rubella was undertaken [2], and reports on the offspring in such cases include only observation at birth. Kamerbeek [11], for instance, reported the results of giving 75-100 ml of rubella convalescent serum to 200 susceptible pregnant women exposed to rubella. None of the women contracted

TABLE 1. *Passive immunization against rubella.*

Reference	Method	No. of individuals	Attack rate %
Barenberg <i>et al.</i> 1942 (4)	Pooled plasma	Not stated	0
Aycock & Ingalls 1946 (3)	Pooled gamma globulin	4 children	No protect.
Stokes 1947 (27)	Pooled gamma globulin 5 ml	5 adults	0
Kamerbeek 1949 (11)	Convalescent serum 75-100 ml	200 pregnant	0
Landon <i>et al.</i> 1946 (16)	Pooled gamma globulin 6 ml	133 children	5
	Controls	129 children	17
Korns 1952 (12)	Pooled gamma globulin 0.1 ml/lb	Children and adults	
	(a) No. 8214-2	199	13
	Controls	201	13
	(b) No. C 3768	53	0
	Controls	52	0
	(c) No. C 3768	45	20
	Controls	60	58
	(d) No. C 3827	38	29
	Controls	46	39
Anderson & McLorinan 1953 (2)	Convalescent gamma globulin		
	(a) 2 ml	424 pregnant	1
	(b) 4 ml	388 pregnant	1
	(c) 4 ml	45 boys	7
	Controls	46 boys	15
	(d) Experimentally induced rubella 4 ml	15 adults	53
	Controls	9 adults	33
Lundström 1953 (18)	Convalescent serum 25-50 ml	98 pregnant	2 (?)
Ward & Parker 1956 (28)	Convalescent serum 30 ml	541 pregnant	0.9
	Controls	102 pregnant	10.8
	Controls	50 not pregnant	12.0
Krugman & Ward 1958 (14)	Experimental infection		
	Normal serum (no antibody)	6 children	83
	Pooled gamma globulin	6 children	17
	Convalescent plasma	6 children	17
	Convalescent gamma globulin	6 children	0
Houser & Schalet 1958 (9)	Pooled gamma globulin 15 ml	300 soldiers	0
	Pooled gamma globulin 5 ml	300 soldiers	0.7
	Placebo	300 soldiers	2.3
Grayston & Watten 1959 (7)	Pooled gamma globulin 5 ml	197 boys	8.5
	Convalescent gamma globulin 2 ml	196 boys	19.2
	Placebo controls	203 boys	16.1
	Uninoculated controls	186 boys	20.4
Present investigation	Convalescent gamma globulin 4 ml	251 pregnant	2.4
	Gamma globulin 24 ml	28 pregnant	0

rubella, 15 (7.5%) had spontaneous abortions, and three infants (1.5%) showed anomalies presumably not belonging to the rubella syndrome. A total of 180 infants ap-

peared healthy at birth, and three pregnancies were not completed at the time of writing.



TABLE 2. *Results of pregnancy in 98 women given 25-50 ml of convalescent serum a ter exposure to rubella (18); follow-up examination of children at 1-3 years of age. The number of offspring is 100, since two pairs of twins are included.*

Result of pregnancy	No. of children following treatment of mothers exposed to rubella in month of pregnancy						Comments
	I	II	III	IV	≥ V	Total	
Spontaneous abortion		2	3			5	<sup>1</sup> Full-term, cause unknown.
Stillbirth		1 <sup>1</sup>	1 <sup>2</sup>			2	<sup>2</sup> Maternal toxæmia.
Neonatal death		2 <sup>3</sup>	1 <sup>4</sup>		2 <sup>5</sup>	5	<sup>3, 5</sup> Premature pairs of twins
Congenital heart disease			1 <sup>6</sup>			1	following maternal toxæmia.
Normal at birth, no follow-up				1	4	5	<sup>4</sup> Birth injury. <sup>6</sup> Probably Fallot's tetralogy.
Normal at follow-up	1	21	19 <sup>7</sup>	21	15	82	<sup>7</sup> One case of funnel-chest, otherwise normal.
Total	1	26	25	22	21	100	

### Treatment with Convalescent Serum

One of us (R.L.) collected a series of 98 pregnant women, with no previous history of rubella and exposed to the disease during an epidemic in Sweden in 1951 [17] and treated with 25-50 ml of rubella convalescent serum [18, 19]. Two women reported a rash some days after treatment, apparently not characteristic of rubella; they were not seen by a physician. Both had normal children. The results of follow-up examinations of the children of prophylactically treated women up to the age of 1 to 3 years are seen in Table 2. Five pregnancies ended in spontaneous abortion and five in perinatal death, four due to obstetrical complications, the fifth being a stillbirth at term (cause unknown). One child was funnel-chested, at follow-up normal after surgery. One child whose mother was pregnant in the third month when treated for exposure to rubella was reported normal at birth, but was found at follow-up to be a blue baby, probably due to Fallot's tetralogy. Five children were not reexamined after birth; one was exposed in the fourth month, the others later in pregnancy. Reexamination of the remaining children did not reveal any abnormalities.

This series, which contains one instance of congenital heart disease as the only anomaly that could be ascribed to the action of inap-

parent rubella despite passive protection, is too small to be conclusive. The incidence of 2% of (questionable) rubella after treatment is low. Previous reports on rubella prophylaxis in pregnancy have not included systematic follow-up studies of the children of the women treated. Consequently, further information on the value of passive immunization against rubella in pregnancy is highly desirable.

An epidemic of rubella in Stockholm in 1955-1956 gave an opportunity for further studies of the subject.

### Case Material

In 1956, the health authorities of the City of Stockholm resolved that susceptible pregnant women were to be given, free of charge, passive immunization against rubella if they were less than 4 months pregnant, and were referred to Kronprinsessan Lovisas Barnsjukhus by the maternity welfare centres, or by private physicians, on account of exposure to rubella. Altogether 65 of the 392 women referred were not treated, for one of the following reasons: exposure had been unlikely; more than 2 weeks had elapsed since exposure; they had been pregnant for more than 4 months; they had an earlier

history of rubella. Immunization was thus given to 327 women. When collecting the series, 48 women were excluded, since—according to the stipulations—they should not have been treated. Thus, 279 definitely exposed women remained for the study; 251 of them were treated with 4 ml of convalescent gamma globulin, and 28 with 24 ml of pooled gamma globulin, since the former was not available during a short period.

The purpose of this study was to examine the children of all the women treated. As five women had emigrated and three could not be traced at the registered address, 271 of the 279 (97.1 %) were included in the follow-up.

Altogether 38 % of the women were housewives without other occupation, 27 % were office personnel, 16 % worked in hospitals or physicians' offices, 12 % were teachers, and 8 % had some other form of work. Seven per cent were less than 20 years, 60 % between 21 and 30 years and 33 % more than 30 years old.

During the epidemic, a number of pregnant women who had contracted rubella were treated with convalescent serum gamma globulin. The outcome of their pregnancies will be reported in a subsequent paper [21].

### Methods

The convalescent serum gamma globulin used in this study consisted of gamma globulin, prepared from the blood of donors who had had an attack of rubella within the previous 4 months. The blood was collected by the National Bacteriological Laboratory, Stockholm, mainly through co-operation with the medical officers of the armed forces. The serum was fractionated according to a modification of Cohn's method 10 by AB KABI, Stockholm. The pooled gamma globulin consisted of similarly produced gamma globulin (KABI) from retroplacental blood collected in maternity hospitals. The dosage was 4 ml of convalescent gamma globulin intramuscularly; when pooled gamma globulin was used, 24 ml were injected by the same route; in both cases the solution was 12 %.

After treatment, the women were given a form to be sent to the investigators, reporting rash if occurring, outcome of pregnancy, name of delivery hospital, and present state of baby's health.

When the children were 2 to 3 years old, they were summoned by us for examination at Kronprinsessan Lovisas Barnsjukhus. Twenty children were examined at other children's hospitals and child welfare centres, or by private practitioners, mainly paediatricians.

Examination of the children was thorough, with special attention to the eyes, ears, heart, and mental development; in the cases examined by the present authors, it included ophthalmoscopy. Children with strabismus and/or other visual disturbances were referred for special ophthalmologic examination. When a heart murmur was present, the ECG and PCG were recorded, and the heart X-rayed. Children suspected of impaired hearing were examined by play audiometry at the Laboratory of Audiology, Karolinska Sjukhuset. When mental retardation was suspected, Terman-Merrill tests were made. The case records of children with complications at delivery and of those admitted to hospital were studied, as were the records of cases reported as abortions.

### Results of Treatment with Convalescent Gamma Globulin

Six of the 251 women contracted rubella despite treatment within 2 weeks of exposure (Table 3). In three of these cases pregnancy resulted in abortion, and one woman gave birth to a child with signs of the rubella syndrome.

*Case 1.* A 25-year-old Para II. Last menstruation Jan. 19, 1956. She was exposed to rubella on March 6, and was given 4 ml of convalescent gamma globulin on March 10. Rubella appeared on March 24; she was given 12 ml of convalescent gamma globulin on the same day. On Oct. 27 she was delivered of a boy weighing 2680 g, and without obvious

TABLE 3. Six cases of maternal rubella after treatment with 4 ml of convalescent gamma globulin, i.e., 2.4 per cent of total 251 women treated.

Result of pregnancy	Rubella in month of pregnancy				Comments
	I	II	III	IV	
Spontaneous abortion		1	2 <sup>a</sup>		Case 1: see text.
Rubella syndrome			1 <sup>a</sup>		
Immature, normal at follow-up			1		
Full-term, normal at follow-up			1 <sup>a</sup>		

<sup>a</sup> One woman also given 12 ml of convalescent gamma globulin after onset of rubella.

defects. On examination at 3 years of age, he was found to show slight mental retardation, hearing impairment, and signs of chorioretinitis.

The other two women who, after treatment, contracted rubella in the third month of pregnancy gave birth to children who were healthy at examination, when they were 2-4 years old.

Of the remaining 245 women who did not contract manifest signs of rubella after treatment, 16 (6.5%) aborted (Table 4).

Severe malformations were present in three children, i.e., congenital cataract, patent ductus arteriosus and mitral stenosis in one case (reported in detail by Malmberg *et al.* [23]), myelomeningocele with internal hydrocephalus in one, and agenesis of the corpus callosum in one case. One case of moderate mental retardation (IQ 79), one of a dermoid cyst and two cases of hypertrophic pyloric stenosis were also recorded.

Among the other children, slight strabismus was recorded in 3 cases without other abnormalities. Minor defects, such as haemangioma of the skin, testicular

hydrocele and inguinal hernia, were considered as unimportant and were therefore not classified as abnormalities.

### Results of Treatment with Pooled Gamma Globulin

Altogether 28 women were treated with 24 ml of pooled gamma globulin (KABI) after exposure to rubella (Table 5). All escaped an attack of the disease. Two women aborted. Two children were not available for examination. One case of mongolism was observed.

*Case 2.* A 25-year-old Para III. Last menstruation April 25, 1956. She was exposed to rubella on June 30, and treated with 24 ml of pooled gamma globulin on July 3. Pregnancy was uneventful. On Feb. 7, 1957, she was delivered of a girl with mongolism. The child was treated in a children's hospital for pharyngitis and mongolism in July 1958, when no other anomalies were detected.

### Discussion

Since rubella is a clinical entity, due with great certainty to a virus infection, protection by passive immunization

TABLE 4. Results of pregnancy (248 children) in 245 women given 4 ml of convalescent gamma globulin after exposure to rubella in first 4 months of pregnancy; none contracted rubella. Repeated exposure and treatment in 12 cases; only 1st exposure listed in table. (Another 6 women contracted rubella despite treatment: see Table 3.)

Result of pregnancy	No. of children following treatment of mothers exposed to rubella in month of pregnancy				
	I	II	III	IV	Total
Spontaneous abortion	—	12	3	1	16
Stillbirth	—	1 <sup>1</sup>	—	3 <sup>2-4</sup>	4
Death	—	1 <sup>5</sup>	3 <sup>6-8</sup>	3 <sup>9-11</sup>	7
Surviving children					
Congenital heart disease	—	—	—	1 <sup>12</sup>	1
Cataract	—	—	1 <sup>13</sup>	—	1
Other abnormalities	—	2 <sup>14, 15</sup>	2 <sup>16, 17</sup>	1 <sup>16</sup>	5
Birth injuries with sequels	—	—	1	1	2
Normal at birth without follow-up	—	2	3	—	5
Birth-weight $\leq$ 2 500 g, normal at follow-up	—	4	2	1	7
Full-term, normal at follow-up	5	56	71	62	194
Three pairs of twins	—	—	4 <sup>18, 19</sup>	2 <sup>20</sup>	6
Total	5	78	90	75	248

<sup>1</sup> Macerated foetus.

<sup>2-3</sup> Birth injuries.

<sup>4</sup> Intrauterine asphyxia.

<sup>5</sup> Toxoplasmosis.

<sup>6</sup> Cataract and VSD.

<sup>7</sup> Meningomyelocele.

<sup>8</sup> Agnesia of corpus callosum.

<sup>9</sup> Capillary bronchitis.

<sup>10-11</sup> Birth injuries.

<sup>12</sup> Minute ventricular septal defect, Roger type.

<sup>13</sup> Small unilateral polar cataract, normal vision.

<sup>14</sup> Dermoid cyst.

<sup>15</sup> Mental retardation: IQ 79.

<sup>16</sup> Hypertrophic pyloric stenosis.

<sup>17</sup> Urinary tract anomaly.

<sup>18</sup> Both twins normal at follow-up.

<sup>19</sup> One twin stillborn macerated; other normal at follow-up.

<sup>20</sup> One twin died of pulmonary atelectasis; other normal at birth, had emigrated at follow-up.

serum or gamma globulin should be theoretically possible. Past experience with varying amounts of convalescent serum and gamma globulin has not given uniform results, as can be seen in Table 1. This may be due to differences in preparation and/or dosage, and/or in the amount of circulating antibodies in the donors. The antibody content cannot be determined in the laboratory. Conse-

quently, clinical observations—with their inherent inaccuracy—are the only means hitherto available for evaluating the efficacy of immunizing procedures against rubella.

In the present series, it was an advantage that the women were referred to a single children's hospital. This allowed control of the indications for treatment, and resulted in only a relatively small

TABLE 5. *Results of pregnancy in 28 women given 24 ml of pooled gamma globulin after exposure to rubella. None contracted rubella.*

Result of pregnancy	No. of children following treatment of mothers exposed to rubella in month of pregnancy					Comments
	I	II	III	IV	Total	
Spontaneous abortion		1	1		2	One case of mongolism Two had emigrated
Abnormalities			1		1	
Normal at birth, not examined later		1		1	2	
Normal at birth and at follow-up		9	9	5	23	
Total		11	11	6	28	

number of cases being lost for the follow-up study.

That the epidemic in question had teratogenic effects has been shown by abnormalities of the rubella type in children observed by us [21], and the cases of rubella deafness reported by Barr & Lundström [5].

Since we could not obtain representative controls—in view of the premises for this survey—the possibility of judging the protective effects by the incidence of manifest rubella after treatment was limited in our series. The incidence of rubella in definitely exposed, susceptible pregnant women was 2.4 per cent (6 of 251). In our opinion, this figure is lower than could have been expected without treatment. The number of exposed women given 24 ml of ordinary pooled gamma globulin is too small to permit any conclusions. It may, however, be recalled that none of these 28 women contracted rubella.

The possibility of attenuating rubella by administration of gamma globulin must be considered, since it is known to have this effect in measles. Inapparent rubella may produce anomalies of the same kind

as the manifest disease. The relatively high incidence of spontaneous abortions, 12 of 78 (15%), when exposure occurred in the second month of pregnancy might support this assumption, even though the overall incidence covering the first four months of pregnancy was only 6.5%. However, only two out of 245 children (0.8%) whose mothers were exposed to rubella and, after treatment with convalescent gamma globulin, apparently did not contract the disease, showed abnormalities belonging to the rubella syndrome. One was in the form of both cataract and congenital heart disease, and the other had a presumably unimportant ventricular septal defect. Compared with the 9% incidence of eye, ear and/or heart defects in a similarly studied material of rubella in early pregnancy in 1951–52 [22], this figure is low, and corresponds far better with the 1.6% of similar anomalies found in the control group of the same study. The occurrence of two gross malformations of the central nervous system is noteworthy but inconclusive, as is one case of mental retardation. The small polar cataract in one case was not of the type seen after maternal rubella.

It is, however, known that defective hearing due to maternal rubella is not infrequent (approximately 20%), when slight hearing loss is included [5]. The incidence of the latter can be estimated only by using audiometric examination in all cases. Since we did not have such facilities, cases of slight hearing loss may not have been recognized in our series.

At the time when this investigation was started, the recommended dose of convalescent gamma globulin for protection of pregnant women was 4 ml [2]. It is possible that a larger dose could have prevented occurrence of the malformations described. Krugman & Ward [14] recommended 10 ml of convalescent gamma globulin or 20 ml of pooled gamma globulin (16%) for the prevention of rubella in early pregnancy. Controlled series with this dosage are not known by us. Even though the dosage used in the present study seemed to afford protection, our results have given reason to advise the higher dosage, and at present 12 ml of (12%) convalescent gamma globulin are administered for prevention of rubella in susceptible pregnant women in Sweden [6]. If such gamma globulin is not available, administration of 24 ml of pooled gamma globulin is recommended for this purpose.

Since it was impossible to obtain controls, no definite proof can be given of the efficacy of passive immunization in our series. The relatively low incidence of rubella in adequately treated women, as well as the extremely few observations of defects possibly due to maternal rubella, are nevertheless encouraging. Consequently, it is recommended that passive immunization of susceptible women, when

exposed to the disease in the first four months of pregnancy, be continued. Additional follow-up studies of the results, with respect not only to the incidence of rubella after treatment, but also to the resulting children up to the age of about 3 years, are therefore desirable.

### Summary

In the present investigation, 251 women—who in 1956 had been exposed to rubella in the first 4 months of pregnancy—were given 4 ml of convalescent gamma globulin. An account is given of a follow-up study, with examination of their children at 2–3 years of age. All but 5 cases were traced. Despite treatment, six women contracted rubella, i.e., an attack rate of 2.4%. Three of these six women aborted, and one had a child with malformations of the rubella syndrome type.

Two of the 245 women showing no signs of manifest rubella had children with defects that might be ascribed to inapparent maternal rubella.

Another 28 pregnant women were given 24 ml of ordinary pooled gamma globulin. None contracted rubella, two had spontaneous abortions, and one gave birth to a child with mongolism.

The present investigation indicates that convalescent gamma globulin and ordinary gamma globulin have a protective effect against rubella.

The following dosage is recommended for future use in pregnant women exposed to rubella. Rubella convalescent gamma globulin (12%), 12 ml i.m. as soon as possible after exposure; when this is not available, pooled gamma globulin (12%), 24 ml i.m.



## References

1. ANDERSON, S. G.: Epidemiological aspects of rubella. *Med J Aust*, **II**: 389, 1950.
2. ANDERSON, S. G. and McLORINAN, H.: Convalescent gamma globulin as prophylactic against rubella. *Med J Aust*, **I**: 182, 1953.
3. AYCOCK, W. L. and INGALLS, T. H.: Maternal disease as a principle in the epidemiology of congenital anomalies. *Amer J Med Sci*, **212**: 366, 1946.
4. BARENBERG, L. H., LEVY, W., GREENSTEIN, N. M. and GREENBERG, B.: Prophylactic use of human serum against contagion in a pediatric ward. *Amer J Dis Child*, **63**: 1101, 1942.
5. BARR, B. and LUNDSTRÖM, R.: Deafness following maternal rubella. Retrospective and prospective studies. *Acta Otolaryng (Stockh)*, **53**: 413, 1961.
6. BLOMQUIST, B., LUNDSTRÖM, R. and THORÉN, C.: Passive immunization against rubella in pregnancy. A follow-up study. (Proc. Ped. Soc.) *Acta Paediat (Upps)*, **49**: 653, 1960.
7. GRAYSTON, J. T. and WATTEN, R. H.: Epidemic rubella in Taiwan 1957-58. III. Gamma globulin in the prevention of rubella. *New Engl J Med*, **261**: 1145, 1959.
8. GREGG, N. M.: Congenital cataract following German measles in the mother. *Trans Ophthal Soc Aust* **3**: 35, 1941.
9. HOUSER, H. G. and SCHALET, N.: Prevention of rubella with gamma globulin. *Clin Research*, **6**: 281, 1958.
10. INGALLS, T. H., BABBOT, F. L., HAMPSON, K. W. and GORDON, J. E.: Rubella. Its epidemiology and teratology. *Amer J Med Sci*, **239**: 363, 1960.
11. KAMERBEEK, A. E. H. M.: Het rubella probleem in het licht van nederlandse ervaringen. *Verh Inst Praevent Geneesk XIV*, Stenfort Kroese, Leiden, 1949.
12. KORN, R. F.: Prophylaxis of German measles with immune serum globulin. *J Infect Dis*, **90**: 183, 1952.
13. KRUGMAN, S. and WARD, R.: The rubella problem. Clinical aspects, risk of fetal abnormality, and methods of prevention. *J Pediat*, **44**: 489, 1954.
14. KRUGMAN, S. and WARD, R.: RUBELLA. Demonstration of neutralizing antibody in gamma globulin and re-evaluation of the rubella problem. *New Engl J Med*, **259**: 16, 1958.
15. KRUGMAN, S., WARD, R., JACOBS, K. G. and LAZAR, M.: Studies on rubella immunization. I. Demonstration of rubella without rash. *JAMA*, **151**: 285, 1953.
16. LANDON, J. F., BASS, M., DAVIDSON, H. B., FOOTE, F. and MUCKENFUSS, R.: Efficacy of gamma globulin in prevention of German measles. *New York J Med*, **5**: 21, 1949.
17. LUNDSTRÖM, R.: Rubella during pregnancy. Its effect upon perinatal mortality, the incidence of congenital abnormalities and immaturity. *Acta Paediat (Upps)*, **41**: 583, 1952.
18. — Passive immunization against rubella during pregnancy. Atti del VI Congresso Internazionale di Microbiologia, Roma, 6-12 Settembre 1953. Vol. 3, Sez. VIII. Pag. 88.
19. — Gamma globulin in Prophylaxe und Therapie bei Infektionskrankheiten. *Neue Oest Z Kinderheilk*, **1**: 193, 1955/56.
20. LUNDSTRÖM, R. and BOSTRÖM, I. L.: Rubella in pregnancy and the incidence of developmental anomalies of the eyes. *Acta Ophthal (Kbh)*, **36**: 782, 1958.
21. LUNDSTRÖM, R., THORÉN, C. and BLOMQUIST, B.: Gamma globulin against rubella in pregnancy. II. Maternal rubella treated with convalescent gamma globulin. A follow-up study. *Acta Paediat (Upps)*, **50**: 453, 1961.
22. LUNDSTRÖM, R.: To be published.
23. MALMBERG, N., STERNER, G. and LUNDMARK, C.: Asymptomatic maternal rubella as a probable cause of embryopathy. *Ann Paediat Fenn*, **3**: 437, 1957.
24. MANSON, M. M., LOGAN, W. P. D. and LOY, R. M.: Rubella and other virus infections during pregnancy. Reports on Public Health and Medical Subjects No. 101. Ministry of Health, Her Majesty's Stationery Office, London, 1960.
25. SCHICK, B.: Diaplacental infection of the fetus with the virus of German measles despite immunity of the mother. *Acta Paediat (Upps)*, **38**: 563, 1949.
26. SIEGEL, M. and GREENBERG, M.: Fetal death, malformation and prematurity after maternal rubella. Results of a prospective study, 1949-1958. *New Engl J Med*, **262**: 389, 1960.
27. STOKES, J., Jr.: The use of gamma globulin from large pools of adult blood plasma in certain infectious diseases. *Ann Intern Med*, **26**: 353, 1947.
28. WARD, H. and PARKER, G.: Passive protection against rubella. *Med J Aust*, **I**: 81, 1956.

Submitted March 4, 1961

Accepted March 10, 1961

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## Gamma Globulin against Rubella in Pregnancy

### II. Manifest Maternal Rubella in Early Pregnancy Treated with Convalescent Gamma Globulin: a Follow-Up Study

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The controversial estimates of the risk of congenital abnormalities have led to different opinions on the management of a woman with rubella in early pregnancy. Some authors have recommended therapeutic abortion [4], whereas others are strictly opposed to an intervention of this type, from religious, medicolegal and/or medical points of view. The matter has recently been discussed by Jeffcoate [6], who found an interruption of pregnancy in such cases acceptable only when a maternal psychiatric indication existed. In any event, induction of abortion is a poor solution of the problem.

Little is known of the possibility of protecting the foetus of a mother with already manifest, early maternal rubella from congenital defects by means of passive immunization. During an epidemic of rubella in Sweden in 1951, a few cases of rubella in pregnancy were treated with 25-50 ml of convalescent serum 1-21 days after appearance of the rash [8]. Three women were pregnant in the third month, four in the fourth month, and two were in later stages of gestation. All nine had full term infants, normal at birth. At examination at 1-3 years of age, eight

were completely healthy; the remaining child could not be traced. However, in view of the small number of observations, the results were inconclusive. By chance, unilateral perceptive deafness was detected in one child—whose mother had been given convalescent serum for rubella in the fourth month of pregnancy—at the age of 5, when the investigation was finished.

Another epidemic of rubella occurred in 1956. Since a number of women with manifest maternal rubella were treated with convalescent gamma globulin, we had an opportunity of making a prospective study of their offspring. The results of prophylactic treatment of pregnant women exposed to rubella have been described separately [5, 10].

#### Case Material and Methods

In 1956, every physician in Sweden who so wished was given, free of charge, 12 ml of convalescent gamma globulin for administration—as soon as possible after appearance of the rash—to each case of rubella in the first 4 months of pregnancy. This dose was three times as large as the 4 ml dose chosen for prophylaxis. Totally 28 women were treated in this way. The average interval

TABLE 1. *Results of pregnancy in 27 cases of maternal rubella given 12 ml of convalescent serum on the average 2.6 days after appearance of the rash.*

Result of pregnancy	No. of cases	Rubella in month of pregnancy				Comments
		I	II	III	IV	
Induced abortion	2		1	1		
Spontaneous abortion	6	1	2	3		One case of missed abortion.
Stillbirth	1			1		Erythroblastosis (Rh-immunization).
Neonatal death	1			1		Immaturity, birth weight 1 520 g
Rubella syndrome	4			4		
Other abnormalities	1				1	Funnel-chest.
Observed at birth only	2			1	1	One normal, one with club-foot.
Normal at 3-4 years	9		1	4	4	
Twin birth	1		1			One twin died at birth, one normal at follow-up.
Total	27	1	5	15	6	

between appearance of rash and treatment was 2.6 days (range 0-7 days). Three of the women also belong to Part I of this study [10], since they had been treated prophylactically shortly after exposure as well. Reports on the outcome of pregnancy and the condition of the children at 3-4 years of age were collected as described in Part I [10]; all but five children were examined by us. Data were available in 27 cases; the remaining woman could not be traced. Two children were observed at birth only; at the follow-up examination, they were living abroad.

### Results

The results of the study are seen in Table 1. Abortion was induced in 3 of the 27 cases studied. One of them was, however, disclosed as a missed abortion. Five women miscarried. If the induced, missed abortion is included, 6 of 25 pregnancies (24%) terminated in early foetal death. In the 19 pregnancies at risk in the first trimester, the abortion rate was 32%. Immaturity at birth was observed in one pair of twins, one of whom died shortly after birth, the other being healthy at

follow-up. Another single immature died on the first day. Autopsy disclosed no anatomic malformations in these cases. Four children with maternal rubella in the third month showed anomalies usually ascribed to maternal rubella, i.e., chorioretinitis and perceptive hearing loss in two cases, chorioretinitis alone in one, and in one case multiple abnormalities including bilateral cataract, congenital bilateral glaucoma, mental retardation, hypospadias and pseudohermaphroditism. One child had a funnel-chest, but was otherwise normal.

When, on one occasion, convalescent gamma globulin was not available, a mother pregnant in the second month was given 40 ml of pooled gamma globulin on the day of appearance of the rubella rash. The resulting infant was a full-term boy, weighing 3050 g, reported normal at birth. At follow-up, feeding difficulties were stated to be present. At 3 years old he showed signs of malnutrition, and had alternating strabismus. Special investigations disclosed no other eye anomalies, nor defects of the ears, heart, or mental development.

### Discussion

When certain virus diseases, e.g. measles, smallpox and chickenpox, occur in a pregnant woman shortly before term, the infant may contract the disease in question after birth, the interval between onset in mother and in child corresponding to the expected incubation period. It is uncertain whether there is an incubation period in early pregnancy as well. Presuming that it does exist, the teratogenic effect might be mitigated by giving antibodies in large quantities as soon as possible after the diagnosis of maternal rubella. Measurable amounts of certain antibodies are, in fact, known to pass the placental barrier as early as in the middle of gestation [14].

Only a few observations on the results of treatment of maternal rubella by passive immunization have been published. Bass [2] reported a pregnant woman who was given gamma globulin after she had contracted rubella; the resulting infant had defects. Berge [3] gave an account of six women with rubella, treated with 75-100 ml of convalescent serum. Spontaneous abortion occurred in two cases, one gave birth to a defective infant, and three had normal infants. In the *Journal of the American Medical Association* 1956 [15] the advice for a pregnant woman with rubella three months and five days pregnant was: "... Gamma globulin should be given to the patient, because it will do her no harm and it may be helpful to the fetus." Spoto [13] recommends passive immunization to all cases of maternal rubella in early pregnancy. Thus, believers in passive immunization in this situation do, in fact, exist.

The outcome of the investigation has shown that, under the circumstances in question, the benefit was apparently none. The high incidence of early foetal death, as well as of abnormalities in the survivors, with great probability due to rubella, are comparable with recent findings of the incidence of abnormalities in prospective studies of maternal rubella not treated by passive immunization [1, 9, 11, 12].

The quantity of convalescent gamma globulin used for treatment of the cases in the present series was three times as large as that used for the apparently successful prophylaxis. Despite this, the unsuccessful outcome of the present trial might be explained by an insufficiently high dose of convalescent gamma globulin. Another possible explanation is that the mechanism of transfer of virus from mother to foetus is not influenced by passive immunization, since passage has already occurred before appearance of the rash in the mother. We cannot count on a neutralization of virus already transmitted to the foetus, since in these early stages no antibody transfer seems to take place.

Judging by our series, administration of 12 ml of 12 % convalescent gamma globulin does not seem to prevent foetal damage in cases of early maternal rubella. It is conceivable that much larger doses would have been effective. We do not, however, believe that there is much likelihood of passive immunization, after appearance of the disease, being successful in preventing such damage. Consequently, the National Bacteriological Laboratory has abandoned the recommendation of convalescent gamma globulin for cases of manifest maternal rubella.

Present resources do not allow a renewed trial with larger amounts of antibodies which, in such case, should be given immediately on onset of the disease.

Rubella virus cannot be propagated for the production of a vaccine. Active immunization by deliberate exposure of young girls to rubella should therefore be encouraged as far as possible. If active immunity has not been acquired by a woman in early pregnancy, exposed to rubella, convalescent gamma globulin or pooled gamma globulin should be administered as soon as possible after exposure.

## Summary

Rubella convalescent gamma globulin was given to 28 women with rubella in early pregnancy. Of those treated in the first trimester, pregnancy terminated in foetal death in 32 % and 11 of 21 offspring survived. At the age of 3-4 years, four of the 21 survivors showed abnormalities belonging to the rubella syndrome. Administration of antibodies to women with manifest rubella in early pregnancy did not succeed in preventing foetal damage.

## References

1. BARR, B. and LUNDSTRÖM, R.: Deafness following maternal rubella. Retrospective and prospective studies. *Acta Otolaryng (Stockh)*, 53: 413, 1961.
2. BASS, M. H.: Recent advances in our knowledge of the contagious diseases of childhood. *Bull NY Acad Med*, 24: 784, 1948.
3. BERGE, B. S. TEN: cit. KAMERBEEK (7).
4. BELL, J.: On rubella in pregnancy. *Brit Med J*, 1: 686, 1959.
5. BLUMQUIST, B., LUNDSTRÖM, R. and THORÉN, C.: Passive immunization against rubella in pregnancy. A follow-up study. (Proc. Ped. Soc.) *Acta Paediat (Upps)*, 49: 653, 1960.
6. JEFFCOATE, T. N. A.: Indications for therapeutic abortion. *Brit Med J*, 1: 581, 1960.
7. KAMERBEEK, A. E. H. M.: Het rubella probleem in het licht van nederlandse ervaringen. *Verh Inst Praevent Geneesk XIV*, Stenfort Kroese, Leiden, 1949.
8. LUNDSTRÖM, R.: Gammaglobulin in Prophylaxe und Therapie bei Infektionskrankheiten. *Neue Oest Z Kinderheilk*, 1: 193, 1955/56.
9. LUNDSTRÖM, R. and BOSTRÖM, I. L.: Rubella in pregnancy and the incidence of developmental anomalies of the eyes. *Acta Ophthal (Kbh)*, 36: 782, 1958.
10. LUNDSTRÖM, R., THORÉN, C. and BLUMQUIST, B.: Gamma globulin against rubella in pregnancy. I. Prevention of maternal rubella by gamma globulin and convalescent gamma globulin. A follow-up study. *Acta Paediat (Upps)*, 50: 444, 1961.
11. LUNDSTRÖM, R.: To be published.
12. MANSON, M. M., LOGAN, W. P. D. and LOY, R. M.: Rubella and other virus infections during pregnancy. Ministry of Health Reports on Public Health and Medical Subjects No. 101, Her Majesty's Stationery Office, London, 1960.
13. SPOTO, P.: Embriopatie da malattie infettive in gravidanza. *Minerva Ginec*, 2: 217, 1950.
14. VAHLQUIST, B.: The transfer of antibodies from mother to offspring. *Advances Paediat*, Vol. X. Yearbook Publishers, Chicago, 1958.
15. QUERIES and MINOR NOTES: Rubella and pregnancy. *JAMA*, 162: 436, 1956.

Submitted March 4, 1961

Accepted March 10, 1961

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## Acute Respiratory Illness and Gastroenteritis in Association with Adenovirus Type 7 Infections<sup>1</sup>

by GÖRAN STERNER, PER GERZÉN, MARIANNE OHLSON and GUNVOR SVARTZ-MALMBERG

Since 1953 adenoviruses types 1, 2, 3, 5, and 7 [9, 13] have been isolated in Sweden. In children adenovirus type 7 can apparently produce acute infections of the upper respiratory tract and in infants atypical pneumonia has been attributed to this virus [4, 9, 11, 13, 16]. No epidemic of type 7 infections has previously been reported in Sweden. However, over the period July-August, 1959, a large number of adenovirus type 7 infections occurred among the population of Nacka, a suburb of Stockholm [8]. In the autumn of the same year such infection was diagnosed in high frequency among the children admitted to two hospitals in Stockholm.

### Materials and Methods

Over the period Aug. 1-Dec. 31, 1959, 717 children (0 to 16 years old) were admitted for acute respiratory infection to the Hospital for Infectious Diseases and the Children's Hospital Samariten. Specimens for virological examination were obtained from 234

of these children. The present investigation was started because an unusually large number of children exhibiting both respiratory and gastrointestinal symptoms were hospitalized. This syndrome has earlier been attributed to adenovirus infections [10]. At the Hospital for Infectious Diseases, specimens for virological examination were obtained from most of the patients with this syndrome but only from a few of those admitted for merely acute respiratory infection. At the Children's Hospital Samariten specimens were taken whether adenovirus infection was suspected or not. Specimens were, however, not obtained from all patients with respiratory symptoms, mainly on account of difficulties in collecting blood specimens (e.g. in infants and in sensitive children).

In addition, 19 children (aged 4 months to 11 years), who contracted acute respiratory illness during their stay in the hospital, were examined, as were all the members of three families in which a child had been found to have infection with adenovirus type 7 (Tables 3 and 4).

To extend the study to a control group, specimens from children who had been admitted for scarlet fever were collected from 33 cases.

Water samples were obtained through the courtesy of the Stockholm Municipal Board of Health. These included raw water from Norsborg water-works, which supply the

<sup>1</sup> The investigation was supported by grants from the Stockholm Municipal Board of Health and from Nils Malmberg's Foundation, which are gratefully acknowledged.

TABLE 1. *Virological data in 234 children admitted with acute respiratory illness (Aug.-Dec. 1959).*

Virus strains isolated from stools	Number of cases	Complement-fixation test against adenovirus
Adenovirus type		
1	1	0/1 <sup>a</sup>
2	7	2/5
3	1	0/0
5	2	0/1
6	1	0/0
7	89	61/82
Coxsackie virus type		
B3	5	0/2
B4	3	1/1
Unidentified cytopathic agents	3	0/2
No virus recovered	110	3/74
No stool specimen obtained	12	6/12
Total	234	73/180

<sup>a</sup> Numerator: number of cases with significant rise. Denominator: number of cases tested.

southern parts of Stockholm and water from an open-air swimming-bath in Lake Mälaren (Mälärhöjdsbadet) in the same area, as well as sewage from apartment houses in the south-western parts of Stockholm, where most of the patients lived.

*Virological methods. Specimens.* Stool specimens for virus isolation were collected from 222 patients as soon as possible after their admission to hospital. From 170 of these patients two or three serum specimens were also taken, the first one on admission and the second one 8-14 days later. In 12 cases paired sera only were obtained (Table 1).

When a nosocomial infection with adenovirus was suspected, stool specimens were collected as soon as possible after the onset of illness, and serum specimens were taken in the acute phase and during the convalescent stage.

As regards the three investigated families, stool specimens and acute-phase sera were

collected on the same day from all the members of each family. Convalescent sera were drawn 14 days later.

In the control group specimens of stool and blood were taken on admission and a second blood sample during convalescence.

*Tissue cultures.* Roller tube cultures of HeLa cells and of monkey kidney cells were used. They were prepared and maintained as previously described [18].

*Virus isolation: Stool specimens.* A 10% suspension was prepared in bovine amniotic fluid to which antibiotics had been added. After low-speed centrifugation 0.1 ml of the supernatant was inoculated into each of three HeLa cell cultures and 0.2 ml into each of three monkey kidney cell cultures. The medium of the HeLa cell cultures was changed after 24 hours' incubation at 35°C and in all cultures after one week. If the cells remained unaffected for 14 days, the isolation test was considered negative. If cytopathic changes occurred in the HeLa cells, the culture fluid was tested for complement-fixation with a pool of human sera from convalescents after adenovirus infection. Materials negative in this test were pooled with fluids from degenerated monkey kidney cultures and passed in HeLa and monkey kidney cell cultures. The fluids from degenerated cultures were again tested for adenovirus antigen.

*Water samples.* One part of phosphate buffered saline of a concentration ten times the physiological one was added to nine parts of the water sample to be tested. The samples were inoculated into HeLa cell cultures, of sewage 1 ml per tube and of other samples 5 ml per tube. The medium was changed 3 hours after inoculation.

*Typing of isolated virus strains.* Adenoviruses were typed by neutralization tests with rabbit hyperimmune sera using the technique devised by Kjellén *et al.* [9]. Delay of cytopathic changes for at least one week was the criterion for type identification. Isolated strains were tested against sera of types 1-17, with the exclusion of types 8 and 12. Rabbit hyperimmune sera were prepared by the method of Kjellén



*et al.* [9] or by a technique using adjuvant immunization [18]. The sera were titrated against a large dose of homologous virus (tissue culture fluid diluted 1:10). A serum dilution that neutralized cytopathic effects for at least 10 days was employed for typing. This dilution, usually 1:2-1:10, did not neutralize other adenovirus types for longer than 4 days at most. To confirm the results new material of the prototype strains 7 'Gomen' and 7A 'S-1058' was obtained from the American Type Culture Collection and a rabbit hyperimmune serum against the Gomen strain from Microbiological Associates, Inc., Washington, D.C.

**Complement-fixation (CF) tests.** The micro-technique used and the preparation of antigen have been described elsewhere [6, 9, 14]. Titres are recorded as the inverted value of the serum dilution. A fourfold or greater rise in titre is considered significant.

**Test for neutralizing antibodies.** Patient sera were tested by the method of Kjellén *et al.* [9] against a type 7 strain (24592/59) which had been isolated during the epidemic. A titre rise was recorded if convalescent serum inhibited the cytopathic effect for at least 3 days more than did acute-phase serum.

**Bacteriological methods.** From 689 children nasopharyngeal, nose and throat swabs for bacteriological examination were taken on admission.

In patients with symptoms of gastroenteritis, stool specimens were cultured for *Salmonella* and *Shigella*, in most infants also for enteropathogenic *E. coli*. Usually several specimens were cultured from each patient.

## Results

**Virological results.** The virological data on the 234 children with acute respiratory illness are summarized in Table 1. Adenovirus strains were isolated from the stools of 101 out of 222 cases investigated for virus excretion. Eighty-nine of these strains were identified as type 7; the others

belonged to types 1, 2, 3, 5, and 6. In addition eight Coxsackie B strains<sup>1</sup> and three agents not yet identified were recovered.

Two of the latter three agents probably belong to the adenovirus group. Thus fluids from the degenerated HeLa cultures of the isolation test contained an antigen capable of fixing complement in the presence of a pool of human sera from convalescents after adenovirus infection. When tested against paired sera, the antigen gave similar titre rises as the standard adenovirus antigen employed. On passage no or only a weak cytopathic effect was observed, although the tissue culture fluid again gave a positive CF reaction. For one specimen (B 8560/59) this CF activity could only be carried through two subpassages. All passages from the other specimen (24642/59) showed CF activity and the cytopathogenicity slowly increased. Only after 10 passages was it pronounced enough for typing tests to be carried out. The strain could, however, not be referred to any of types 1-7, 9-11 or 13-17. It should be mentioned, that a second stool specimen obtained from the same patient 19 days later than 24642/59 was shown to contain adenovirus type 1.

Paired sera were obtained from 82 of the 89 patients excreting adenovirus type 7. Sixty-one of them showed a rise in CF titre against adenovirus antigen. Of the other 21 children all but two had a titre of at least 4. Eight had, in both sera, titres of 32 or higher. In most of these 8 cases acute-phase serum had been taken later than 1 week after the onset of illness.

Only a few specimens were examined for the presence of neutralizing antibodies against adenovirus type 7. It should be

<sup>1</sup> In 3 of the Coxsackie cases a significant rise in neutralizing antibodies was demonstrated. From the other 5 cases one serum specimen only was obtained, but all these specimens were found to contain neutralizing antibodies to the isolated virus type.



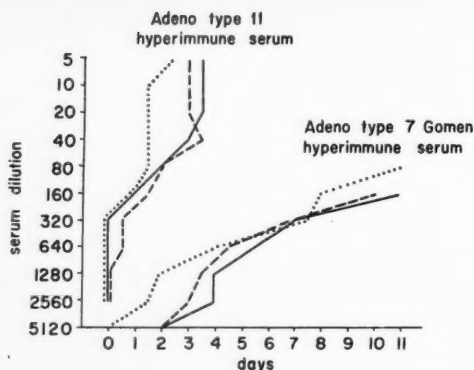


Fig. 1. Neutralization of adenovirus type 7 'Gomen' and type 11 'Slobitski' hyperimmune sera against type 7 'Gomen', type 7A 'S-1058' and strain B 8543/59 isolated during the observed epidemic. Abscissa: duration of inhibition of cytopathic changes. Ordinate: inverse value of serum dilution. Three tissue culture tubes per dilution step. —, prototype 7 'Gomen' received from Dr. Huebner 1956 (inoculation dose  $10^{5.2}$  TCID<sub>50</sub>). ---, prototype 7A 'S-1058' received from ATCC 1960 (inoculation dose  $10^{4.5}$  TCID<sub>50</sub>). ····, strain B 8543/59 (inoculation dose  $10^{4.2}$  TCID<sub>50</sub>).

mentioned, however, that the two patients, who excreted adenovirus type 7 without developing a response in CF titre ( $<2-4$ ;  $2-2$ ) both had neutralizing antibodies against adenovirus type 7, one of them with a rise in titre. On the other hand, only one of three patients with titre rise in CF antibodies but negative stool specimen had neutralizing antibodies (and in addition a titre rise) against type 7. Finally, rises in CF as well as in type 7 neutralizing antibody titres were demonstrated in 6 out of 12 patients from whom paired sera but no stool specimen were obtained.

The only patient excreting Coxsackie virus who showed rising CF titre ( $<2-4$ ) against adenovirus had no neutralizing antibodies against type 7.

The results of the family studies and of the investigations of the cases with nosocomial infection are summarized in Tables 3 and 4.

In none of the 33 controls with scarlet

fever could adenovirus be isolated. Nor could a rise in CF titre be demonstrated.

No virus was found in raw water from the water-works or in the water of the open-air swimming-bath, whereas adenovirus type 7 was demonstrated in sewage from an apartment house in one of the suburbs south-west of Stockholm and Coxsackie virus type B 3 from another house in the same district.

According to some authors [12], a subtype 7A can be established within the type 7 group. The results obtained in our laboratory by repeated neutralization tests agree more closely with those obtained by Binn *et al.* [3], in that only insignificant differences could be demonstrated between the prototype strains 7A and three strains from the current epidemic. As an example the results of an experiment with a hyperimmune serum against the Gomen strain are shown in Fig. 1. Analogous results were obtained with another line of the Gomen strain,

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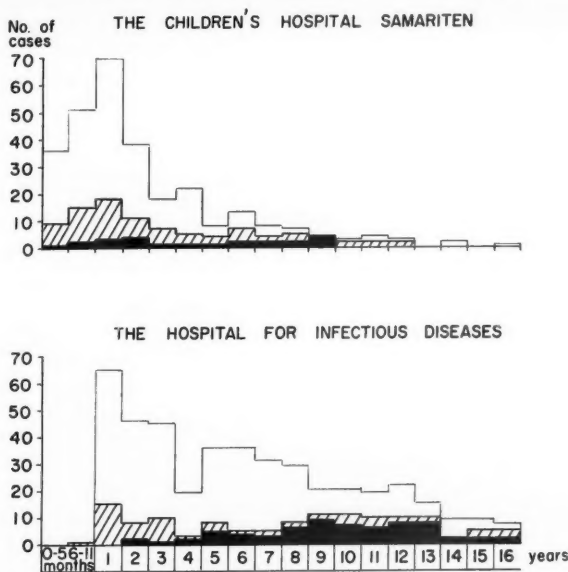


Fig. 2. Age-distribution of 717 children admitted for acute respiratory illness during Aug.-Dec. 1959. ■ Adenovirus type 7 isolated. ▨ No adenovirus type 7 isolated. □ Stool specimen not obtained.

which for control purposes was imported from the American Type Culture Collection in 1960, as well as with another Gomen antiserum which was obtained from Microbiological Associates, Inc. In accordance with earlier observations [3, 17] hyperimmune sera against types 3, 11 and 14 were found to inhibit temporarily the cytopathic effect of type 7 strains. This phenomenon was most pronounced for serum against type 11. Fig. 1 illustrates the marked difference in slopes between the curves representing heterotypic and homotypic neutralization. The prototype strains and three current strains tested behaved identically in this respect.

**Bacteriological results.** The bacteriological results are of special interest in those cases where the nose and throat swabs were col-

lected before any antibiotic therapy was started. Within this group one or more of the following bacteria were found in about 50 per cent of the virologically investigated children and about 60 per cent of those not investigated virologically:  $\beta$ -haemolytic streptococci, *Staph. aureus*, pneumococci and *Haemophilus influenzae*. The frequency of  $\beta$ -haemolytic streptococci was higher in the children who had not been investigated virologically. This is explained by the fact that during the late-autumn epidemic of scarlet fever (Fig. 3) in Stockholm a great number of children with streptococcal tonsillitis were admitted to the Hospital for Infectious Diseases, and these were not investigated virologically. Among the patients at the Children's Hospital Samariten a higher frequency of pneumococci was found than among those at the Hospital for Infectious Diseases. This difference is possibly attributable to the age-distribution of the patients (Fig. 2), since findings of pneumo-

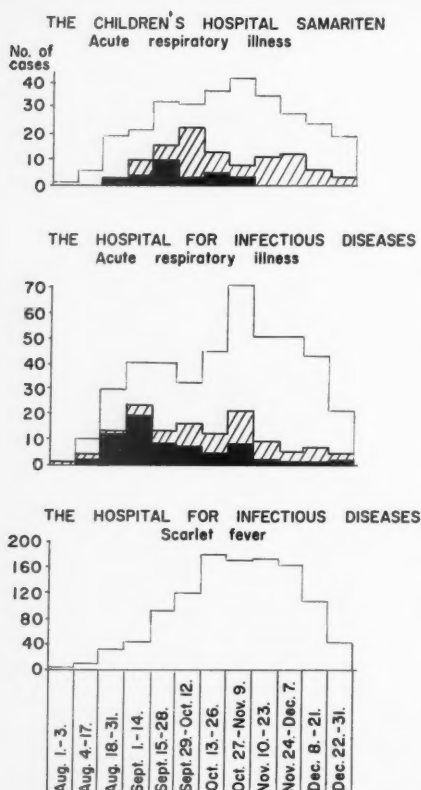


Fig. 3. Number of children admitted with acute respiratory illness or scarlet fever by 14 day periods. ■ Adenovirus type 7 isolated. ▨ No adenovirus type 7 isolated. □ Stool specimen not obtained.

cocci in the nose and throat are reported to be most common in the youngest age-groups [15]. Concerning the other bacteria mentioned no particular differences were disclosed.

One or more of the above-mentioned bacteria was similarly found in about 50% of those 45 children with acute adenovirus type 7 infections, who had not received antibiotic therapy before collection of specimens. The number of cases is too small, however, to allow a definite assessment of the frequency of the different bacterial species.

In children who had received antibiotics—in most cases penicillin—before collection of specimens, the frequency of the above-named bacteria, *Haemophilus influenzae* excepted, was lower.

From the 253 patients virologically investigated, no *Salmonella*, *Shigella* or enteropathogenic *E. coli* were recovered. In the same age-group altogether 10 cases of *Salmonella* infection, 3 cases of *Shigella* infection and 1 case of infection with enteropathogenic *E. coli* were diagnosed at the two hospitals during the period under investigation.

**Clinical observations.** On the basis of clinical symptoms the material can be divided into two main groups: patients with acute respiratory illness, and patients with respiratory illness combined with gastrointestinal symptoms (Table 2).

Of patients investigated virologically 138 belong to the first group. They exhibited symptoms referable to nose and throat, in some cases even to the lower respiratory tract. In 29 of the latter patients bronchopneumonia was diagnosed by X-ray. Symptoms of laryngotracheitis were rare. No conclusions should be drawn from this fact, however, since such patients are as a rule admitted to otological wards at other hospitals.

In the second group, consisting of patients who, besides symptoms referable to the respiratory tract, exhibited gastrointestinal symptoms—diarrhoea with or without vomiting for more than 1 day—84 (including 3 cases of bronchopneumonia) were investigated virologically.

Patients with gastroenteritis—excepting infants—are as a rule admitted to the Hospital for Infectious Diseases. The total number of children with *only* gastroenteritis who were admitted to this hospital

TABLE 2. *Distribution of adenovirus type 7 isolations from cases with different clinical syndromes.*

Symptoms	The Children's Hospital Samaritan			The Hospital for Infectious Diseases		
	Number of cases			Number of cases		
	Total	Investigated	Adenovirus type 7 isolated	Total	Investigated	Adenovirus type 7 isolated
Acute respiratory illness with no signs of gastroenteritis	262	84	19	334	54	17
Acute respiratory illness with gastroenteritis	26	11	4	95	73	49
Gastroenteritis with no signs of acute respiratory illness	19	0	0	25	5	0

over the period August–December, 1959, was only 25, of whom 13 were found to be infected with *Salmonella* (10 cases) or *Shigella* (3 cases). On the other hand, 95 children with respiratory as well as gastrointestinal symptoms were admitted. Seventy-three of these were investigated virologically and adenovirus type 7 was recovered from 49.

Fig. 2 shows that adenovirus type 7 was isolated from children of all age-groups including infancy (the youngest patient being 4 months old). The greatest number of strains were recovered from patients at the age of 7–10 years. All the infants, except one, were admitted to the Children's Hospital Samaritan.

The peak incidence of admitted cases occurred in September (Fig. 3).

Most children from whom adenovirus type 7 was isolated were admitted on the third or fourth day of illness.

It has earlier been shown that adenovirus can be excreted for a long time after the onset of infection [9, 10]. For this reason the detailed clinical study will be limited to patients with evidence of acute adenovirus type 7 infection: either a po-

sitive stool specimen and a rise in titre of CF or type 7 neutralizing antibodies or a rise of both kinds of antibodies but negative or no stool specimen. This makes

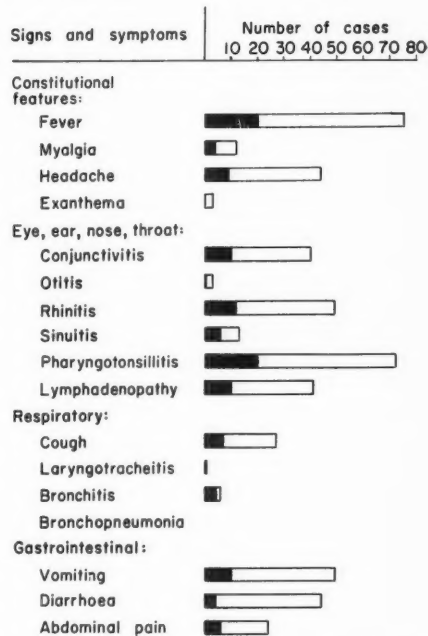


Fig. 4. Clinical features in 75 children with acute adenovirus type 7 infections. ■ = children treated at the Children's Hospital Samaritan.

a total of 75 cases from both hospitals, including 6 cases of hospital infection in children admitted for some other illness. As seen in Fig. 4, the main symptoms were fever, conjunctivitis, rhinitis, pharyngotonsillitis, cervical lymphadenitis and in more than half of the cases gastroenteritis. An uncharacteristic exanthema was noted in three children. In more than four-fifths of the cases the fever ( $\geq 37.4^{\circ}\text{C}$ ) started with a sudden rise to  $39\text{--}40^{\circ}\text{C}$  and lasted for 2 to 12 days; only two children had fever of less than 5 days' duration. The conjunctivitis was often bilateral; only in a few cases was it severe and of a purulent nature. The rhinitis was usually fairly mild. Nearly all children had pharyngotonsillitis. Plugs in the tonsils were often observed, also in cases where  $\beta$ -haemolytic streptococci were not found; many of these cases had, however, received antibiotics before the collection of specimens. In about half of the cases the cervical lymph-nodes were moderately enlarged. In a few children the course was complicated by acute otitis media. The diagnosis of sinusitis was made only if pathological changes in the nasal sinuses could be demonstrated radiologically. Out of 24 examined children only 13 exhibited such changes of varying degree of severity, from thickening of the mucous membranes to complete occlusion. Cough was noted in 27 children. X-ray examination of the lungs, performed in 25 children including five who had bronchitis, showed no abnormalities.

Nausea and vomiting were common. In more than half of the cases diarrhoea was the predominating feature and led to admission to the Hospital for Infectious Diseases on the suspicion of *Salmonella* infection. However, these children had in

addition signs of respiratory infection. Marked abdominal pain was not unusual, whether other symptoms of gastroenteritis were present or not.

Many of the children complained of headache, a few of giddiness as well. Muscle pains, mostly in the extremities, were present in some cases. Signs of lesions of the central nervous system were not seen. Examinations of the cerebrospinal fluid in 17 children revealed no abnormalities as regards cell, protein or sugar content. The general condition was in most children good, despite the high fever, and the period of convalescence was usually short.

Electrocardiograms were taken in 62 children. All but one were normal. The pathological one showed prolongation of the P-R interval and was from a child infected with  $\beta$ -haemolytic streptococci in addition to adenovirus type 7.

Out of 69 non-nosocomial cases 39 had on admission white blood cell counts below and 10 above the range given as normal for the age [5]. Values below 4000 were recorded in 12 children, whereas only two had counts exceeding 15,000. A slight or moderate increase in neutrophil leucocytes was recorded in most cases. The erythrocyte-sedimentation rate (E.S.R.) was determined according to Westergren (in 59 cases) or by Ström's micromethod (in 10 cases). The highest recorded value (mm per hour) varied from normal to 47 (Westergren) and 43 (Ström). In several of the children with a white blood cell count exceeding 10,000 or high E.S.P. or both, bacterial complications could not be demonstrated. All the children had haemoglobin values of at least 11 g per 100 ml and normal urine findings.

Among those children from whom adenovirus type 7 was isolated without serological evidence of acute adenovirus infection there also occurred a syndrome of

TABLE 3. *Clinical and virological data among the members of three families in which a child has been found to have infection with type 7 adenovirus on admission to the Children's Hospital Samariten.*

+, obvious; (+), slight; -, absent

	N. Family					Kj. Family				T. Family			
Age (years)	7♀	2♀ <sup>a</sup>	30♂	9♂	29♀	2♀ <sup>a</sup>	35♂	32♀	10♂	7♂ <sup>a</sup>	5♀	28♀	35♂
Day of onset	Sept. 11	16	23	26	—	Sept. 17	Oct. 6	9	12	Oct. 22	30	30	Nov. 2
Max. temp. (°C)	40.0	40.4	38.8	39.0	—	40.0	38.5	38.0	—	39.4	40.4	39.5	38.6
Duration of fever (days)	7	13	7	7	—	7	3	3	—	8	5	4	6
Cephalalgia	—	—	—	+	—	+	—	—	—	+	—	+	+
Myalgia	—	—	—	—	—	+	+	+	—	—	—	—	—
Conjunctivitis	+	(+)	+	+	—	+	—	+	—	—	+	+	+
Rhinitis	+	+	(+)	(+)	(+)	+	+	+	+	—	—	—	—
Pharyngotonsillitis	+	+	—	+	—	+	—	(+)	—	+	+	+	(+)
Cough	+	+	—	+	—	—	—	—	—	—	—	—	—
Bronchopneumonia	—	+	—	—	—	—	—	—	—	—	—	—	—
Gastroenteritis	—	—	—	—	—	—	—	—	—	+	—	—	—
Adenovirus type 7	+	+	+	+	+	+	—	+	+	+	—	+	+
CF titre Serum I	2	128	4	4	4	< 2	4	< 2	8	16	16	16	4
Serum II	8	128	4	32	64	4	4	4	8	64	8	16	16

<sup>a</sup> Hospitalized.

fever, conjunctivitis, pharyngotonsillitis and, in many cases, gastroenteritis. This group included 5 cases of pneumonia, in one of which the patient was a 2 year old girl who had severe atypical pneumonia complicated by myocarditis (see Table 3, family N).

All the children excreting adenovirus types 1, 2, 3, 5 and 6 were ill with fever and pharyngitis; one child from whom type 2 was isolated had, in addition, radiologically verified pneumonia. One of the children who had adenovirus type 1 in her stools was probably the source of a type 1 infection in another child in the same ward.

The patients from whom Coxsackie virus was isolated had fallen ill with respiratory symptoms. Some had, in addition, muscle pains and mild diarrhoea.

*Epidemiology.* Prior to the outbreak of the epidemic described here 19 adenovirus strains were recovered from children admitted to the Children's Hospital Samariten and the Hospital for Infectious Diseases in Stockholm over the period Jan. 1958–July 1959 (276 investigated cases). Of these strains seven belonged to type 7 and the others to types 1, 2, or 5. In July, 1959, an adenovirus type 7 epidemic occurred at Nacka, a suburb south-east of Stockholm [8].

Other infectious diseases that occurred epidemically in Stockholm in 1959 were influenza and scarlet fever. Thus an influenza B outbreak took place during January–March. Absence rates in the schools of up to 25% were noted during January–February [1], after which they fell to normal in the last part of March



TABLE 4. *Virological data in 19 children with nosocomial acute respiratory illness.*

Virus strains isolated from stools	Number of cases	Complement-fixation test against adenovirus
Adenovirus type		
1	1	1/1 <sup>a</sup>
2	1	0/1
7	10	5/7 <sup>b</sup>
No virus recovered	7	0/5
Total	19	6/14

<sup>a</sup> Numerator: Number of cases with significant rise. Denominator: Number of cases tested.

<sup>b</sup> One case with CF titres < 2.2 had rise in neutralization test against adenovirus type 7.

(about 8%). In the autumn no cases of influenza were recorded. Only sporadic cases of enterovirus infections (polio, Coxsackie and ECHO viruses) occurred in 1959. While the investigation presented here was in progress, a large epidemic of scarlet fever broke out. It will be seen from Fig. 3 that the incidence of scarlet fever reached its maximum somewhat later than did that of the adenovirus type 7 infections.

The Hospital for Infectious Diseases receives patients from the whole of Stockholm, whereas the Children's Hospital Samariten covers mainly the south-western parts of Stockholm and of its surroundings. Most of the above-described type 7 cases came from the southern and the western suburbs where the number of large families is greater than in any other residential area of Stockholm. Multiple cases with similar symptoms frequently occurred in the home, in the apartment house, among playmates, in day-nurseries, in the schools. The family study summarized in Table 3 shows that virtually symptom-free infections also occurred.

On the suspicion of hospital infection with adenovirus, 19 children were investigated. Adenovirus type 7 was isolated from 10 (Table 4). Serological results suggested that the infection was contracted in the hospital in at least 6 of these cases. That this type of infection is readily communicable is demonstrated by the following chain of nosocomial infections, which also illustrates the difficulty in drawing aetiological conclusions from the clinical picture in acute respiratory illness.

1. *S. record no. 1687/59.* A 9 year old boy who on Dec. 8, 1959, became ill with temperature of 40°C, sore throat, mild conjunctivitis, vomiting and profuse diarrhoea. Penicillin treatment at home. Admitted on Dec. 10. Nasopharyngeal culture: *H. influenzae*. Repeated cultures from stools: normal bacterial flora. Stool specimen on Dec. 11: adenovirus type 7. CF antibodies against adenovirus: Dec. 11: < 2, Dec. 19: 32.

2. *S. record no. 1689/59.* A 2 year old boy who was admitted on Dec. 11, the day after patient no. 1, with whom he shared room for the next 24 hours only. For a few days he had had high fever, purulent conjunctivitis and mild diarrhoea. Penicillin treatment at home without effect. Bronchopneumonia, otitis and sinusitis diagnosed on admission. Nasopharyngeal culture: *H. influenzae*. Afebrile on Dec. 15 but remained in hospital for social reasons. Dec. 21 (11th day at hospital): Temperature 39.2°C. Recurrence of conjunctivitis. Intense rhinopharyngitis. Loose stools on a few occasions. X-ray of chest on Dec. 29: regression of earlier changes. Nasopharyngeal culture on Dec. 28: *H. influenzae*. Stool specimen on Dec. 12: no cytopathic agent; Dec. 28: adenovirus type 7. CF antibodies against adenovirus on Dec. 12: 2, Dec. 30: < 2, Jan. 7, 1960: 8.

3. *S. record no. 1698/59.* A 2 year old girl who had had high spiking fever, rhinitis, cough, and conjunctivitis. Admitted on Dec. 12. Placed with patient no. 2 in a two-bed room. X-ray of chest: extensive areas of



bronchopneumonia on both sides. Nasopharyngeal culture: *H. influenzae*. Afebrile on Dec. 14. Stayed for further investigation of chest deformity. Dec. 30: Temperature 39.8°C, rhinopharyngotonsillitis, enlarged cervical lymph-nodes. No conjunctivitis. Stools normal. Nasopharyngeal culture on Jan. 2, 1960: *H. influenzae*, and scanty growth of *Staph. aureus*. Stool specimen on Dec. 13, 1959: no cytopathic agent; Jan. 3, 1960: adenovirus type 7. CF antibodies against adenovirus: Dec. 12, 1959: 16; Jan. 2, 1960: 16, Jan. 11:  $\geq 64$ . Neutralizing antibodies against type 7: Dec. 13, 1959: negative, Jan. 2, 1960: negative and Jan. 11: + +.

From these case records it will be seen that patient no. 1 probably spread the infection to patient no. 2, who on admission had no adenovirus infection. This patient probably was the source of the infection that patient no. 3 contracted during her stay in the hospital.

The incubation period is estimated to 7-10 days.

### Discussion

The present study of an epidemic of adenovirus type 7 infections in Stockholm in the autumn of 1959 should permit more definite conclusions concerning some clinical features of such an infection than most previous studies have allowed. In most respects the picture in the 75 children with proved acute infection with adenovirus type 7 agrees well with that earlier described for such infections: high and sometimes spiking fever for 1 week with acute symptoms referable to the nose and throat, conjunctivitis and moderate enlargement of the cervical lymph-nodes. In the epidemic under investigation there occurred, in addition, a great number of cases with symptoms of gastroenteritis.

During the period of investigation *Salmonella* and *Shigella* infection in children occurred to a small extent only. On the other hand, from children with gastroenteritis in combination with respiratory symptoms adenovirus type 7 was isolated in about two-thirds of the investigated cases. The incidence of isolated adenovirus type 7 strains was actually higher among children with this combination of symptoms than among children with respiratory infection only, in whom about one-third of those investigated excreted this virus type. No adenovirus infections were demonstrated in a control series consisting of patients with scarlet fever from a period of time that practically coincided with the height of the adenovirus epidemic and from the same parts of Stockholm.

It seems therefore established that adenovirus type 7 does not only cause respiratory symptoms but can also give rise to gastroenteritis in children. Previous findings have suggested that adenovirus type 3 may also produce gastrointestinal symptoms [2, 10]. The limited family study showed that virtually symptom-free type 7 infections may also occur.

The high incidence of adenovirus type 7 infections among the hospital patients indicates that there was an epidemicspread, particularly in those large-family residential areas south and west of Stockholm, from where most of the patients came. The spread of adenovirus type 7 within the families as well as the hospital infections suggest that the virus spreads readily in closed groups. The general morbidity during the adenovirus type 7 epidemic was, however, not at all so high as, for instance, during epidemics of influenza

and did not exceed the average morbidity among school-children at this time of the year [7].

Modes of transmission, other than personal contact, for instance, via children's paddling pools, public baths, etc., are possible but have not been established by evidence.

### Summary

During an epidemic in Stockholm in the autumn of 1959 adenovirus type 7 was isolated from 99 hospitalized children. There was serological evidence of acute adenovirus infection in 75 cases. The virus strains could not be referred to types 7 or

7A according to the scheme of Rowe *et al.*, since no definite differences between the prototypes for these strains could be demonstrated by cross-neutralization tests. In addition to the symptoms of acute respiratory illness observed previously in such infections, many of the patients exhibited symptoms of gastroenteritis, for which no other cause could be established.

### Acknowledgements

The authors' thanks are due to Dr G. Tunevall for supervising the bacteriological examinations, and Mrs M. Brundin, Mrs U. Grundel, Mrs V. Josefsson and Miss U. Petersson for technical assistance.

### References

1. Skolöverläkarens för Stockholm årsrapport för 1958-1959, bilaga 1.
2. BARR, J., KJELLÉN, L. and SVEDMYR, A.: Hospital outbreak of adenovirus type 3 infections. *Acta Paediat*, 47: 365, 1958.
3. BINN, L. N., HILLEMANN, M. R., RODRIGUEZ, J. E. and GLABERE, R. R.: Antigenic relationships among adenoviruses with appraisal of reliability of complement fixation test for typing isolates. *J Immunol*, 80: 501, 1958.
4. CHANY, C., LÉPINE, P., LELONG, M., LETAN-VINH, SATGÉ, P. and VIRAT, J.: Severe and fatal pneumonia in infants and young children associated with adenovirus infections. *Amer J Hyg*, 67: 367, 1958.
5. FANCONI, G. and WALLGREN, A.: Lehrbuch der Pädiatrie. Benno Schwabe & Co, Basel/Stuttgart 1961.
6. FULTON, F. and DUMBELL, K. R.: The serological comparison of strains of influenza virus. *J Gen Microbiol*, 3: 97, 1949.
7. HJÄRNE, U.: Personal communication.
8. KALLINGS, L. O. and MADSEN, B.: To be published.
9. KJELLÉN, L., STERNER, G. and SVEDMYR, A.: On the occurrence of adenoviruses in Sweden. *Acta Paediat*, 46: 164, 1957.
10. KJELLÉN, L., ZETTERBERG, B. and SVEDMYR, A.: An epidemic among Swedish children caused by adenovirus type 3. *Acta Paediat*, 46: 561, 1957.
11. OKER-BLOM, N., WAGER, O., STRANDSTRÖM, H., MÄKELÄ, P. and JANSSON, E.: Adenoviruses associated with pharyngoconjunctival fever. *Ann Med Exp Fenn*, 35: 342, 1957.
12. ROWE, W. P., HARTLEY, J. W. and HUEBNER, R. J.: Serotype composition of the adenovirus group. *Proc Soc Exp Biol Med*, 97: 465, 1958.
13. STERNER, G.: Infections with adenovirus type 7 in children and their relationship to acute respiratory disease. *Acta Paediat*, 48: 287, 1959.
14. SVEDMYR, A., ENDERS, J. F. and HOLLOWAY, A.: Complement fixation with the three types of poliomyelitis viruses propagated in tissue culture. *Amer J Hyg*, 57: 60, 1953.
15. TUNEVALL, G.: Oto-rhinological infection in childhood. *Acta Paediat*, 41: Suppl. 92, 1952.
16. TYRRELL, D. A. J., BALDUCCI, D. and ZAIMAN, T. E.: Acute infections of the respiratory tract and the adenoviruses. *Lancet*, 271: 1326, 1956.
17. UCHIDA, S., HOSHIKA, T., YAMAMOTO, H., KOIKO, K., KOSEKI, S. and FURUNO, A.: Relationships between types of adenovirus indicated by heterologous neutralization. *Jap J Exp Med*, 29: 21, 1959.
18. v. ZEPEL, G. and SVEDMYR, A.: A study of the association of ECHO viruses to aseptic meningitis. *Arch Ges Virusforsch*, 7: 355, 1957.

Submitted Dec. 9, 1960

Accepted Dec. 16, 1960

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## The Bacterial Flora of the Upper Respiratory Tract and Gut in Children of Nomad Lapps<sup>1</sup>

by GUNNAR LAURELL and TORE MELLBIN

The nomad Lapps differ in certain respects from the rest of the population in Sweden. They are constantly on the move, following the reindeer herds high up into the mountain districts during the summer and down towards the coast in winter, and the families therefore have to have at least two, and often more, dwelling places. During the summer these people are isolated in the mountains; in winter they usually live in small inland villages. Compared with the rest of the population, therefore, they are in most respects more isolated all the year round. This isolation has been reduced during recent years, especially in the southern regions, but in the most northerly districts it is still marked.

Like other children in Sweden, the nomad children begin school at 7 years of age. Owing to the great distances they go to boarding schools, where they are in close contact with each other, in contrast to the time before they start school and to the summer holidays.

The contact that exists between the nomad school-children and the sedentary population varies in the different school

districts, and is closer in the more southerly of those investigated. The children from the northernmost districts are therefore more isolated during both term and holidays, and also before they start going to school.

The life of the Lapps is, as we have said, conditioned by the movements of the reindeer. The proximity of the people to these animals and their produce and the abundance of dogs might be expected to be reflected in the bacterial flora of the people.

With regard to these conditions, investigations were carried out to discover the nature of the bacterial flora of the nomad children in connexion with other investigations on these children (Mellbin, to be published).

An investigation such as this is also of interest in a wider sense. In these times there are some difficulties in finding material to make up a normal series. This applies not least to the bacteria of the respiratory tract. The widespread use of antibiotics has produced a shift in the bacterial flora, sensitive organisms being eliminated and replaced by resistant, sometimes of the same species but commonly of another. This trend is most

<sup>1</sup> This investigation was supported by a grant from the County Council of Norrbotten, Sweden.

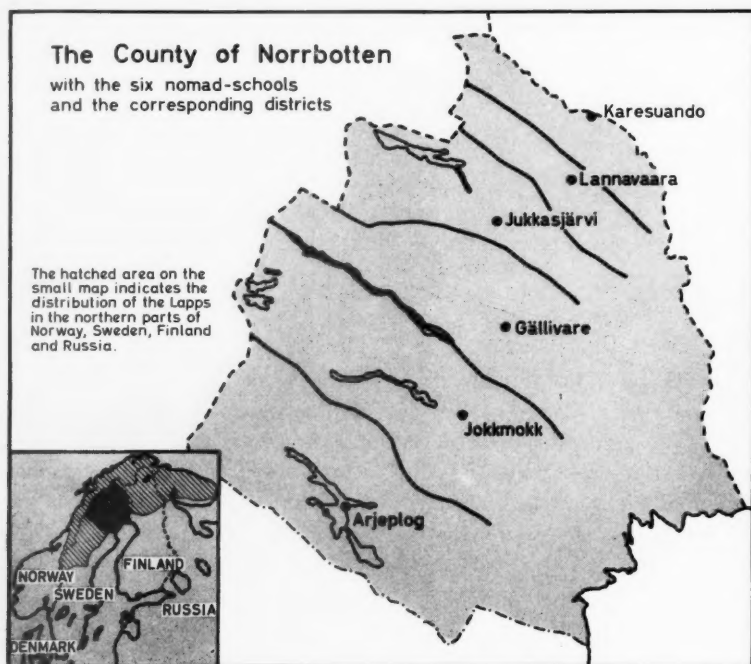


Fig. 1.

marked in hospitals and close communities but is also encountered in standard sections of the population. During recent years, therefore, attempts have been made to find material in which the bacterial flora is unspoiled by antibiotics. Searches have been made among populations living under isolated conditions. Particular attention has been paid to *Staphylococcus aureus*, and in 1954 Hopps, Wisseman & Whelan (4) investigated skin-strains isolated from Dusums in northern Borneo and from patients in tropical Mexico. All were penicillin sensitive, but some formed penicillinase. An investigation that also illustrates the incidence of *Staph. aureus* in the respiratory tract was per-

formed in 1956 by Rountree (7). She examined 120 persons from the Wagaba region of New Guinea, and demonstrated *Staph. aureus* among 23 of them. All strains were sensitive to penicillin, streptomycin, oxytetracyclin, chlortetracyclin, and chloramphenicol. Phage-typing revealed that the types present were the same as those encountered in the respiratory tracts of persons in civilized regions.

Although the isolation of the Swedish Lapps can in no way be compared to that of the populations above-mentioned, it is fairly marked.

To obtain an all-round conception of the bacterial flora, the organisms taken up in this investigation include not only

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*Staph. aureus* but also other latent pathogens commonly found in the respiratory tract and gut.

### Material

The series comprises all children at the nomad schools in Karesuando, Lannavaara, Jukkasjärvi, Gällivare, Jokkmokk, and Arjeplog in the County of Norrbotten (see Fig. 1). The children were aged 7-14 years.

Samples were collected in autumn 1957 and autumn 1958. During the first period of investigation swabs were taken from nose and throat, specimens of faeces were collected, and serum was taken for serological analysis from the 55 children in Form 1 immediately on arrival at school, in order to obtain results not affected by the new environment. Seven weeks later the same tests were made on all 330 children in Forms 1-7 [1-6]. During the second period of investigation, in autumn 1958, nose and throat swabs were taken from all 310 children immediately on arrival at school.

Because teaching in Form 7 is concentrated to two (Jukkasjärvi and Gällivare) of the six nomad schools, the composition of the series differs slightly from the first occasion to the second. On the first occasion the children in the highest form are classed with the school they had been attending for the past 7 weeks; on the second the seventh-form children are classed with the school they had attended during the previous year, as the particular school environment is unimportant in this connexion.

No detailed investigations with typing or serological analysis were carried out during the second period of investigation. The tests using special transport-substrate were performed in both 1957 and 1958.

### Methods

#### Collection of samples

Three different swabs were taken from the upper respiratory tract. Two were taken with

ordinary swab-sticks from the antrum nasi and throat, respectively. The third was taken with a naso-pharynx swab. The swab-sticks were immediately placed in oblique-agar tubes, and dispatched by the fastest possible means to the Institute of Bacteriology, Uppsala. A new transport substrate described by Stuart, Toshach & Patsula (8) was tried on each occasion, certain of the samples from throat and naso-pharynx being duplicated. These were sent with the rest of the samples.

*Specimens of faeces* were dispatched in ordinary plastic tubes, with no added substance.

Specimen-taking was arranged to fit in as closely as possible with train time-tables. The mean interval between sampling and incubation was 30 hours, and was equal for specimens from schools in both northern and southern districts.

Blood samples for *serological analysis* were collected by venipuncture. They were allowed to stand for 4-6 hours to coagulate, after which the tubes were centrifuged. The serum was pipetted off, immediately frozen, and stored at -20°C until required.

### Culture

The bacteriological specimens were cultured on the following substrates.

#### Respiratory tract

1, Agar with 10% sheep's blood. 2, Agar with 10% sheep's blood and gentian violet (1/750,000). 3, Phenol-mannite agar with 7.5% NaCl (Chapman) (1). 4, Haematin agar. 5, Sabouraud agar. 6, Ordinary meat broth with 7.5% NaCl for enrichment of *Staph. aureus*. The swabs from the antrum nasi were inoculated on this medium. 7, Ascites broth with sodium azide (1/4000) for enrichment of *Pneumococci*.

#### Faeces

8, Ordinary endoagar. 9, Conradi Drigalski for typing *E. coli*. 10, Phenol-mannite agar with 7.5% NaCl (Chapman) (1). 11,

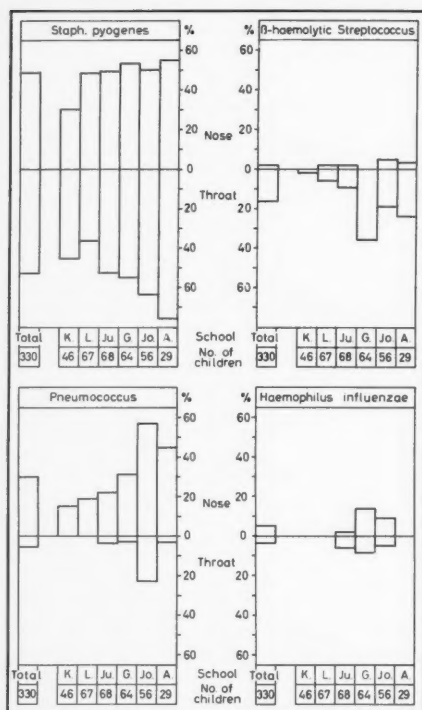


Fig. 2. Pathogenic bacteria. Percentage incidence in nose and throat among children from the various schools. Schools: K. = Karesuando, L. = Lannavaara, Ju. = Jukkasjärvi, G. = Gällivare, Jo. = Jokkmokk, A. = Arjeplog.

Kauffman enrichment medium for isolation of *Salmonella*. 12, Desoxycholate citrate agar plates for *Salmonella* and *Shigella*.

Plates 1, 3, 4, and 5, and tubes 6 and 7 were incubated in air at 37°C, and were read off after 48 hours. Plates 3 and 5 were then incubated for a further 3 days at room temperature, and again read off. The gentian violet plates were incubated in air for 48 hours. Secondary culture was carried out from tubes 6 and 7, if no growth was obtained on the primarily inoculated plates.

When reading off cultures from the air passages all suspected *Staph. aureus*,  $\beta$ -

haemolytic *Streptococci*, *Pneumococci*, *H. influenzae*, and fungi were noted. The suspect colonies were then examined as follows.

*Staphylococci*. The coagulase test was carried out, and all coagulase-positive strains, irrespective of pigment formation, were subsequently referred to as *Staph. pyogenes*. Most strains obtained at the first period of investigation were phage-typed by Dr. Gösta Wallmark, at the National Bacteriological Laboratory, Stockholm. One hundred and fifty-five strains obtained on the same occasion were also tested for formation of  $\alpha$ -,  $\beta$ -, and  $\delta$ -lysin, using the technique worked out by Elek & Levy (2).

*Streptococci* were tested on soluble haemolysin. Strains forming free haemolysin were grouped and typed by Dr. Ingmar Juhlin, at the Department of Bacteriology, Allmänna Sjukhuset, Malmö.

*Pneumococci* were typed by Neufeld's capsular swelling reaction. Untypable *Pneumococci* were confirmed by means of optochin (strip method).

*H. influenzae* were confirmed by means of the satellite phenomenon.

*Fungi*. Isolated fungi were obtained in pure culture, and typed in the usual manner by means of staining techniques, culture on spore substrate, and biochemical fermentation.

The examination of the faeces included standard tests for *Salmonella* and *Shigella* organisms. Pathogenic *E. coli* were selected via Conradi Drigalski medium, and were confirmed by standard serological tests. *Staph. pyogenes* were isolated from phenol-mannite plates.

#### Sensitivity tests

All strains of *Staph. pyogenes* recovered at the first period of investigation were tested by the disk method (3). The limits between sensitivity and resistance were set as follows: penicillin, 2 I.U./ml; erythromycin, 4  $\mu$ g/ml; streptomycin, 12  $\mu$ g/ml; tetracyclines, 4  $\mu$ g/ml; chloramphenicol, 8  $\mu$ g/ml; novobiocin, 16  $\mu$ g/ml. In the description that follows



TABLE 1. *Children with positive throat and/or nasal culture at the various schools during the two periods of investigation (1957, after 7 weeks at school; 1958, beginning of term). Percentage incidence.*

School	Karesu- ando	Lanna- vaara	Jukkas- järvi	Gälli- vare	Jokk- mökk	Arje- plog	Total
First period of investigation, 1957							
No. of children	46	67	68	64	56	29	330
<i>Staph. pyogenes</i>	59	61	66	78	73	86	69
$\beta$ -haemolytic							
<i>Streptococcus</i>	2	8	10	36	23	28	17
<i>Pneumococcus</i>	15	18	24	33	68	48	33
<i>Haemophilus influenzae</i>	0	0	7	20	13	0	8
Fungi	7	8	10	13	13	14	10
Second period of investigation, 1958							
No. of children	46	62	60	49	61	32	310
<i>Staph. pyogenes</i>	74	84	78	61	70	91	76
$\beta$ -haemolytic							
<i>Streptococcus</i>	4	3	0	2	2	6	3
<i>Pneumococcus</i>	43	50	28	24	21	38	34
<i>Haemophilus influenzae</i>	11	8	2	8	3	0	5
Fungi	43	24	35	37	36	22	33

strains that were inhibited by these concentrations are termed sensitive, and the rest resistant.

#### Serological tests

*Antistreptolysin titres (AST)* were determined chiefly as described by Ipsen (5). The results, however, are given in the manner proposed by Packalén & Bergqvist for antistaphylolysin (ASta) determinations (6).

*Antistaphylolysin titres (ASta)* were determined in accordance with the directions of Packalén & Bergqvist (6).

#### Results

##### Upper Respiratory Tract

##### *Staph. pyogenes*

##### Incidence

Fig. 2 shows the incidence of *Staph. pyogenes* in throat and nose among the children at the various schools during the first period of investigation. The total percentage incidence of this organism in

TABLE 2. *Percentage incidence of Staph. pyogenes, Pneumococcus, and fungi among first-form children on starting school and after 7 weeks at school. The corresponding figures for the whole series, 1957 and 1958, are included for purposes of comparison.*

Organism	First form 1957		Total series	
	on starting school, 55 children	after 7 weeks at school, 55 children	1957 330 children	1958 310 children
<i>Staph. pyogenes</i>	93	60	69	76
<i>Pneumococcus</i>	31	40	33	34
Fungi	53	13	10	33



TABLE 3. *Staph. pyogenes*. Phage patterns among 302 strains isolated from nose and throat during the first period of investigation.

Phage group	Phage pattern	First form on starting school, 55 children	Total series after 7 weeks at school, 330 children
I	52/52A/KS6	1	10
	52/52A/166	0	2
	52/52A/80	2	2
	KS6	0	21
	52/80	2	1
		5	36
II	3A	3	13
	3B/3C/55/71	6	15
	3B/3C	0	1
	3C/55	0	3
	3C/55/71	0	3
	71	6	46
		15	81
III	6/47/53	0	4
	6/47/54/75/819/1034+	2	9
	53	0	3
	53/54	0	3
	73	4	3
	77	0	3
	Further 16 patterns	5	19
		11	44
IV	42D+	1	1
		1	1
I + III	52A/79/53/73/819	0	4
	Further 2 patterns	1	2
		1	6
	166/155	1	20
	155	0	7
		1	27
Untypable		32	41
Total number of strains		66	236

the respiratory passages is illustrated in Table 1. *Staph. pyogenes* was isolated from 69 % of all the children, the local figures showing considerable variations (from 59 % in Karesuando to 86 % in Arjeplog). The incidence of Staphylococci after the period of 7 weeks at school was thus lower in the more northerly schools than in those farther south. This difference is significant.

Scrutiny of the figures for the incidence of Staphylococci among school starters (see Table 2), from whom swabs were taken both at the beginning of term and 7 weeks later, reveals that 93 % of these children had Staphylococci in the nose and/or throat on the first occasion, whereas the corresponding figure at 7 weeks was 60 %. Because the series is so small (55 children), it cannot be divided

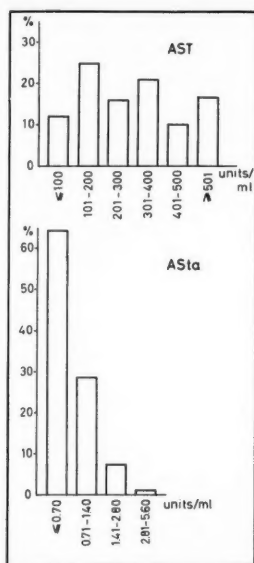


Fig. 3. Antistreptolysin (AST) and antistaphylo-lysin (ASta) titres. Percentage distribution.

up into the respective school groups, but 31 of the children that had *Staphylococci* at the beginning of term still had these organisms, 20 had lost them, and 2 that had previously been negative now, after 7 weeks, had acquired them. Two children showed negative cultures on both occasions.

At the 1958 examination (see Table 1) all specimens were taken at the beginning of term. The overall incidence of *Staph. pyogenes* was then 76%, and there was no clear difference between the northern and southern schools.

#### Sensitivity tests

Altogether 364 strains were tested. The incidence of strains resistant to most antibiotics was extremely low, amounting to 0.8% for erythromycin, chlortetracycline, oxytetracycline, tetracycline, and chlor-

amphenicol, and to 1.1% for streptomycin. The incidence of strains resistant to penicillin was 7.9%, and to novobiocin 5.5%. The figure concerning novobiocin is noteworthy, especially as it was found that 29% of the *Staphylococci* strains isolated from the school starters at the beginning of term were resistant to this substance.

#### Phage typing

A total of 302 strains were examined, 66 from children in the first form at the beginning of term, and 236 from all children 7 weeks after the beginning of term. The results are shown in Table 3. Phage typing was on the whole successful, especially in the case of strains isolated 7 weeks after the commencement of term. Of these latter 236, only 41 proved to be untypable, whereas the corresponding figures for the first-form children at the beginning of term were 32 out of 66 strains, i.e. 48%. After 7 weeks at school 32 strains of *Staph. pyogenes* were isolated from the first-formers, and of these only three (9%) were untypable. The typable strains were chiefly referable to groups I, II, and III, with nearly twice as many in group II as in any of the other three groups. It is particularly interesting that no fewer than 52 strains belonged to phage type 71. Apart from the higher incidence of untypable strains, there were no differences concerning distribution between strains isolated at the beginning of term (school-starters) and those isolated 7 weeks later (whole series).

#### Haemolysin pattern

Altogether 155 strains of *Staphylococci* were examined, and the results are given

TABLE 4. *Staph. pyogenes*. Haemolysin pattern for 155 strains isolated during the first period of investigation.

Haemolysin pattern	Number of strains	Percentage distribution	Elek & Levy percentage distribution of 200 strains
$\alpha\beta\delta$	26	17	11
$\beta\delta$	2	1	—
$\alpha\delta$	105	68	82
$\alpha$	9	6	3
$\delta$	13	8	4
Total	155	100	100

in Table 4. It is of interest that 18 % showed  $\beta$ -haemolysis, which is more than what is usually found in Staphylococci isolated from man.

#### *Antistaphylolysin (ASta) determinations*

Serological examination (see Fig. 3 and Table 5) disclosed no important incidence of raised ASta titres. The titre in no fewer than 92 % was 1.4 U. or less. Concerning the raised titres, 7 % of the children had titres between 1.4 and 2.8 U., and only 0.9 % exceeded 2.8 U. There was no significant difference between the various schools, or between the age groups. The mean value for the series was 0.85 U. (limits 0.74 U. in Jokkmokk and 0.98 U. in Karesuando).

#### *$\beta$ -haemolytic Streptococci*

##### *Incidence*

During the first period of investigation Streptococci were isolated from 57 children (17 %). As can be seen from Fig. 2 and Table 1, the variations were fairly great. In the two northernmost schools these organisms were isolated in only 2 % and 8 % of children, respectively, whereas the incidence in Gällivare was 36 % and in the two southernmost schools 23 % and 28 %. They were chiefly isolated from the throat, but nasal carriers were also found. The incidence of the organism among school starters at the beginning of term was 9 %. During the second period of investigation the overall incidence of  $\beta$ -haemolytic Streptococci was only 3 %, with isolated cases in five of the six schools.

TABLE 5. *Antistaphylolysin and antistreptolysin titres. Mean values at the different schools.*

School	Karesu- ando	Lanna- vaara	Jukkas- järvi	Gälli- vare	Jokk- mokk	Arje- plog	Total
No. of children	46	67	68	64	56	29	330
Antistaphylolysin (ASta), mean titres	0.98	0.83	0.90	0.84	0.74	0.83	0.85
Antistreptolysin (AST), mean titres	348	375	484	395	268	199	334

*Typing*

The results can be seen in Table 6. Typing could be carried out satisfactorily, and most of the strains belonged to Lancefield's group A. Other groups were represented by only a few strains. Type 9 was commonest (19 strains), followed

TABLE 6. *Streptococcus*. Type-distribution among 69 strains isolated from nose and throat during the first period of investigation.

Type	First form on starting school, 55 children	Total series after 7 weeks at school, 330 children
Group A		
6	0	8
9	1	18
10	0	8
12	1	3
16	0	2
21	1	5
27	0	1
28	0	2
Group B	0	1
Group E	0	1
Group F	0	2
Group G	1	1
Group L	0	2
Untypable	0	8
Not typed	1	2
Total no. of strains	5	64

by types 6 and 10. It is of interest that four strains belonged to type 12, a type ascribed importance in connexion with nephritis.

*Antistreptolysin (AST) determinations*

The results of serological analysis are collected in Fig. 3 and Table 5. A surprisingly large number of high titres were noted, no fewer than 26 % exceeding 400 U. There were fairly large variations between the schools, with significantly lower mean values for the 85 children from the two southernmost schools despite the higher

incidence of Streptococci at these. If the titres are arranged according to age groups, it is found that the mean titres increase over the three lowest forms (286, 329, 367), and then remain roughly constant at the high mean level of 398. No correlation between these figures and the current incidence of  $\beta$ -haemolytic Streptococci could be established.

The AST values for 54 school-starters were rather higher at the beginning of term (mean 310) than after 7 weeks at school (mean 286). After this period the titres for 30 children had fallen, 13 were unchanged, and 11 had increased.

*Pneumococci**Incidence*

The incidence of Pneumococci during the first period of investigation was 33 % (see Fig. 2 and Table 1). Here again there were considerable variations between the schools, the two northernmost showing the lowest values (15 % and 18 %, respectively), and the two southernmost showing the highest figures (68 % and 48 %, respectively). At the beginning of term Pneumococci were present in 31 % of the school starters, and 7 weeks later in 40 % (see Table 2).

During the second period of investigation the incidence was 34 %, the variations from school to school were less, and no statistical difference could be found between the northernmost and southernmost schools.

*Typing*

Typing of Pneumococci was carried out during the first period of investigation, and here again most strains were typable (only 5 % proved to be untypable). No

predominating type was noted. Type 19 was commonest, followed by types 23, 18, and 39.

The typability was poorer among strains isolated from school starters at the beginning of term. Of 17 strains it was possible to type only nine (53%), whereas all strains isolated from the same children 7 weeks later were typable.

### *Haemophilus influenzae*

#### *Incidence*

This organism was isolated during the first period of investigation in 8% of all children (see Fig. 2 and Table 1). The variations were great, and in several schools *H. influenzae* was not present at all. On the second occasion the overall incidence was 5%, and again the variations were great. Typing was not undertaken.

### *Fungi*

#### *Incidence*

During the first period of investigation fungi were isolated from 10% of the children after 7 weeks at school (see Table 1). The incidence was lowest in Karesuando, and increased southwards to a maximum of 14% (Arjeplog). Specimens taken from school starters at the beginning of term (see Table 2) showed that 53% of the children then had fungi in throat and/or nose, whereas the incidence of these organisms in the same children 7 weeks later was 13%, an incidence of the same order of magnitude as that for the larger series. This difference is highly significant.

During the second period of investigation the incidence of fungi was 33%, with fairly even distribution between the vari-

ous schools and age groups (except for Forms 1 and 2, in which the figures were 50% and 40%, respectively).

#### *Typing*

During the first period of investigation typing of 60 strains was carried out. Of these 43 were *Candida albicans*, 6 *Geotrichum*, 4 *Torulosis*, 3 *Trichosporon*, 2 non-pathogenic *Cryptococcus*, 1 *Candida parakrusei*, and 1 *Rhodotorula*.

### *Faeces Culture*

#### *Incidence of organisms*

No *Salmonella* or *Shigella* bacteria were isolated, but *Staph. pyogenes* and pathogenic *E. coli* were present. The findings are collected in Table 7. *Staph. pyogenes* was isolated in 41 cases, i.e. 12%, and was commonest in Jokkmokk. Pathogenic *E. coli* were isolated in eight cases. In five of these the strain was 0111B4, and the remaining three were 0125B15; 0126B16; and 0112a, 0112c.

### *Transport Medium*

Having regard to the special interest attached to an improved transport medium, a new substrate previously used chiefly for Gonococci was tried, first tentatively and during the second period of investigation to a greater extent. The results are shown in Table 8. As can be seen, the medium offers certain advantages in the case of the most sensitive organisms, namely *Haemophilus influenzae*. No particular advantages were apparent with regard to other bacteria. It is worthy of note that fungi were isolated more than twice as often with ordinary agar tubes as when the special substrate was used as

TABLE 7. *Faeces culture. Distribution of positive cultures among the various schools.*

School	Karesu- ando	Lanna- vaara	Jukkas- järvi	Gälli- vare	Jokk- mökk	Arje- plog	Total
No. of children	46	67	68	64	56	29	330
<i>Staph. pyogenes</i>	6	9	1	6	16	3	41
Pathogenic <i>E. coli</i>	0	1	4	2	0	1	8

transport medium. The same trend was apparent, though less markedly, in the case of *Staph. pyogenes*.

### Discussion

The incidence of *Staph. pyogenes* was surprisingly high during both periods of investigation, viz. 69 and 76 %, respectively; and an incidence of as much as 93 % is recorded for the school starters. As a rule the incidence of Staphylococci outside hospital is about 40 %, and in 1954 among the natives of New Guinea it was only 19 %.

Striking too is the fact that the incidence

of Staphylococci is greater among children arriving at school from the isolated home environment than after 7 weeks' life at school in close contact with other children in classrooms and dormitories. The high incidence in these children at the beginning of term may be connected with their proximity at home to domestic animals, from which these organisms may originate. When they leave the home environment, the incidence of Staphylococci falls.

On phage-typing it was found that 48 % of the school-starters' Staphylococci at the beginning of term were untypable, whereas the corresponding figure after 7 weeks was only 9 %. This would support

TABLE 8. *Distribution of positive and negative cultures of the organisms concerned, using different media.*

Organism	Culture	First period of investigation		Second period of investigation	
		Throat		Nose and throat	
		Agar	Special	Agar	Special
<i>Staph. pyogenes</i>	Negative	2	20	20	125
	Positive	58	40	325	220
$\beta$ -haemolytic <i>Streptococcus</i>	Negative	6	1	4	3
	Positive	9	14	5	6
<i>Pneumococcus</i>	Negative	2	5	50	42
	Positive	7	4	67	75
<i>Haemophilus influenzae</i>	Negative	5	0	12	3
	Positive	0	5	9	18
Fungi	Negative	0	9	12	61
	Positive	10	1	95	46

the theory that the Staphylococci originate from domestic animals.

It is of interest that many of the strains belonged to phage-type 71, a type described as particularly common in impetigo among children. Oddly enough, it is not especially common in the respiratory passages of children with this infection. There was no case of impetigo among the children that were carriers of this phage-type.

The incidence of  $\beta$ -haemolysis among the isolated Staphylococci was 18%, which is higher than that usually noted in Staphylococci isolated from man;  $\beta$ -haemolysis is commoner among Staphylococci from animals, on the other hand (2).

Despite the high incidence of *Staph. pyogenes*, the antistaphylolysin titres were within normal limits in 92% of the children, and only 0.9% showed undoubtedly raised titres. The ASta titres were of the same order of magnitude at the beginning of term and 7 weeks later in the school-starters.

The organisms showed marked sensitivity to most of the antibiotics tested. Of the strains 7.9% were resistant to penicillin, and the incidence of strains resistant to novobiocin was 5.5%. The high figure for penicillin may be due to previous treatment with this substance: it is known that a resistant strain may persist long after it first invaded the respiratory passages. The novobiocin-resistant strains are not so easily explained, since this antibiotic has with absolute certainty never been used among this population. There may be some connexion with the fact that these Staphylococci to some extent originated from animals. It is not known whether animal Staphylococci have

a naturally higher resistance to novobiocin, but the fact that 29% of the strains isolated from the school-starters at the beginning of term were resistant to novobiocin would be in favour of this.

Whereas the incidence of Staphylococci during the second period of investigation (at the beginning of term) was fairly even at the various schools, it is found that during the first period of investigation, which took place 7 weeks after the beginning of term, the incidence increased the farther south the school was situated. As mentioned in the introductory paragraphs, the more southerly schools are less isolated from the sedentary population than are those in the north. The higher incidence of Staphylococci in the more southerly districts may therefore be due to closer contact with carriers from outside the schools.

The incidence of  $\beta$ -haemolytic *Streptococci* during the first period of investigation varied greatly from one school to another. The reason for this is probably cross infection among the children during the 7 weeks they had been resident at school before the swabs were taken. The lower incidence at the beginning of term, both among school-starters during the first period of investigation and in the total series during the second (9% and 3%, respectively), would support this assumption. Whether the spread within the groups may be explained by the presence of carriers among the children themselves or by infection from members of the sedentary population it is impossible to say, but it is a fact that the incidence of Streptococci is much lower in the northern, more isolated schools.

Typing yielded nothing of special

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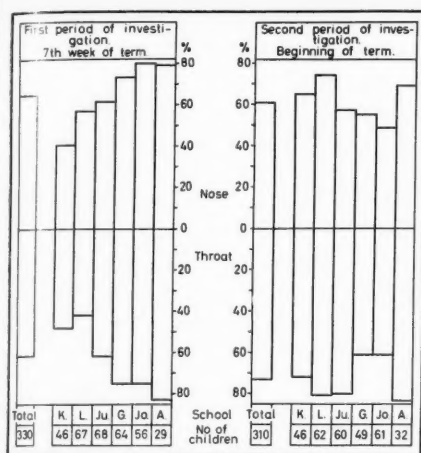


Fig. 4. Percentage incidence of infection with one or more of the pathogens demonstrated during the two periods of investigation. Schools: K. = Karesuando, L. = Lannavaara, Ju. = Jukkasjärvi, G. = Gällivare, Jo. = Jokkmokk, A. = Arjeplog.

interest, apart from the fact that four strains belonged to type 12. This is regarded as particularly liable to cause nephritis, but no clinical signs of this condition or of rheumatic infection were seen.

It is difficult to find a satisfactory explanation for the unexpectedly high anti-streptolysin titres, which exceeded 400 U. in 26% of cases. The rise in titre with increasing age is in accordance with the findings in earlier investigations. The high titres among the children from the more northerly schools may possibly be due to the lower social standard prevailing in those districts. Owing in part to the poor housing conditions, respiratory infections are common, especially during winter, before the children even start school and in school holidays.

*Pneumococci.* Pneumococci were com-

mon, and were isolated to the same extent during both periods of investigation. As in the case of Staphylococci, the incidence was higher in the more southerly schools at the first examination, but on the second occasion the distribution was even. The typability appeared to be poorer among the strains introduced into the environment by the children themselves than among those isolated 7 weeks after the beginning of term.

The incidence of *Haemophilus influenzae* was low on both occasions, and varied greatly from school to school. No conclusions can be drawn from the figures available. It is, however, of interest to note that it was at all possible to isolate these comparatively sensitive organisms in spite of the long time that elapsed between sampling and culture.

The influence of carriers of infection among the sedentary population upon the children residing in boarding schools is indicated in Fig. 4. The percentage incidence of infection with one or more of the pathogens demonstrated in nose and throat after 7 weeks at school shows an obvious increase from north to south, which clearly reflects the degree of contact between the school-children and the rest of the population. For purposes of comparison, the corresponding figures for the second period of investigation are included. At that time the children had just returned from a stay of about 3 months' duration in the mountains. The high overall incidence of bacteria on this occasion is explained by the higher incidence of Staphylococci among the children at the beginning of term (see Table 1).

The incidence of *fungi* in swabs taken immediately on the children's arrival at

school was remarkably high. The results of both periods of investigation are in this respect equal: 53 % of the school-starters on the first occasion and 50 % of the first-formers during the second period of investigation were carriers of fungi. The incidence was highest among the youngest children, but even the older ones showed an incidence of 29 % at the beginning of term. This high incidence fell for the school-starters during the next 7 weeks to 13 %. As with the Staphylococci, the most obvious explanation would be that the fungi originated from domestic animals at home, and disappeared after the contact with these ended.

*Examination of the faeces* revealed a higher incidence of both *Staph. pyogenes* and pathogenic *E. coli* than is usual in a normal series. The high incidence of Staphylococci is probably connected with the abundance of these organisms in the respiratory tract. No child had diarrhoea at the time of sampling.

*The special substrate* that was tried appeared to offer advantages with regard to the isolation of *H. influenzae*. These bacteria were recovered in very much greater numbers during both periods of investigation from this substrate than from the standard media. In the case of other organisms, especially Staphylococci, the result was the reverse.

### Summary

The incidence of bacteria in the respiratory passages and gut of Lapp children

attending the six nomad schools in the County of Norrbotten, Sweden, was investigated during autumn 1957 and autumn 1958.

The incidence of *Staph. pyogenes*, possibly to some extent originating from domestic animals, was high, particularly on the arrival of the children at the schools. This was also true of fungi. The Staphylococci were highly sensitive to most of the antibiotics against which they were tested.

The antistaphylolysin titres (ASTa) were almost always within normal limits, only 0.9 % of the children showing titres over 2.8 U.

$\beta$ -haemolytic Streptococci were present in more of the children after 7 weeks at school than at the beginning of term. This would indicate cross-infection.

The antistreptolysin titres (AST) were remarkably high, 26 % of the children showing values exceeding 400 U.

The incidence of Pneumococci and *H. influenzae* varied greatly from one school to another.

The overall incidence of bacteria after 7 weeks at school was higher in the more southerly schools, which might be due to infection from the sedentary population.

Faeces culture resulted in fairly abundant growth of *Staph. pyogenes*, and pathogenic *E. coli* were isolated in eight cases.

A new transport medium was tried. This gave enhanced results with *H. influenzae*, but less prolific growth of *Staph. pyogenes* and fungi.

## References

1. CHAPMAN, G. H.: The significance of sodium chloride in studies of staphylococci, *J Bact*, 50: 201, 1945.
2. ELEK, S. D. and LEVY, E.: Distribution of haemolysins in pathogenic and non-pathogenic staphylococci. *J Path Bact*, 62: 541, 1950.
3. ERICSSON, H., HÖGMAN, C. and WICKMAN, K.: A paper disc method for determination of bacterial sensitivity to chemotherapeutic and antibiotic agents. *Scand J Clin Lab Invest*, 6, Suppl. 11: 21, 1954.
4. HOPPS, H. E., WISSEMAN, C. L., JR. and WHELAN, J.: Relation of antibiotic resistance of staphylococci to prevalence of antibiotic therapy in diverse geographic areas. *Antibiot Chemother*, 4: 270, 1954.
5. IPSEN, J.: A standard for antistreptolysin O of human serum and its practical application. *Acta Path Microbiol Scand*, 21: 203, 1944.
6. PACKALÉN, T. and BERGQVIST, S.: Staphylococci in throat and nose and antistaphylococcal titre. *Acta Med Scand*, 127: 291, 1947.
7. ROUNTREE, P. M.: Staphylococci harboured by people in western highlands of New Guinea. *Lancet*, 1: 719, 1956.
8. STUART, R. D., TOSHACH, S. R., PATSULA, T. M.: The problem of transport of specimens for culture of gonococci. *Canad J Publ Hlth*, 45: 73, 1954.

Submitted February 15, 1961

Accepted February 20, 1961

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## Immune Electrophoretic Studies of Bovine Milk and Milk Products

by LARS Å. HANSON and I. MÅNSSON

A large number of antigenic substances have been demonstrated in bovine milk by means of immune electrophoresis (5, 6, 8). Some 10-15 of these antigenic factors are related to blood serum proteins, among others to the albumin and the  $\gamma$ -globulin. Several of the specific milk proteins have been identified with chemically characterized proteins such as  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, casein and red protein. The immunological techniques provide a more descriptive and complete picture of the milk proteins than does, for example, simple electrophoresis. For this reason we have used the immune electrophoretic technique to study the changes of the proteins which may be induced by various methods employed in the commercial preparation of milk and milk products. The possibility of using this immunological method for the analysis of milk allergies is also taken into consideration.

### Material

#### Antigens

Bovine colostrum taken two hours after parturition was used as well as mature milk taken more than three weeks after parturition. Merthiolate was added to a concentration of 1/10,000. These materials were also

examined after heat-treatment for 15 minutes at 55°, 60°, 65°, 70°, 75°, 80°, 85°, 90°, 95°, 100° and 120°C. Mature milk was analyzed after being subjected to the following pasteurization procedures: 63°C for 30 minutes, 75°C for 19 seconds and 90°C for 120 seconds.

Dried milk prepared by various methods<sup>1</sup> (spray-dried, single and double drum-dried) was examined as well as samples of commercially available baby-food products<sup>2</sup> containing milk, spray-dried after pre-heating to 84°C. A commercial preparation of bovine milk advertized as "non-allergenic" and intended to be given to infants hypersensitive to bovine milk<sup>3</sup> was also tested. For the analyses the milk powders were dissolved in the phosphate buffer (pH 8.4) used for the electrophoresis.

#### Immune sera

Anti-colostrum immune sera were prepared in rabbits by weekly injections of 2 ml of colostrum for 4 weeks. After this time they were bled. Other bleedings were taken one week after subsequent booster doses. Comparison of immune electrophoretic analyses of milk with the anti-colostrum sera and with anti-mature milk immune sera showed mainly identical spectra. The anti-colostrum

<sup>1</sup> Kindly supplied by Eng. Thorsell, VMC, Vänersborg (Sweden).

<sup>2</sup> "Semper Välling" and "Baby Semper" kindly supplied by SMP, Stockholm (Sweden).

<sup>3</sup> "Allergilac", Cow-lac Ltd., London (England).

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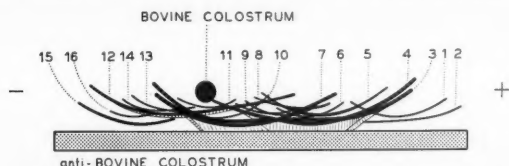


Fig. 1a. Diagrammatic representation of an immune electrophoretic analysis of bovine colostrum by means of anti-colostrum immune serum. The following precipitation lines are identified: 2 and 3, casein; 4,  $\beta$ -lactoglobulin; 5, serum albumin; 7,  $\alpha$ -lactalbumin; 8, red protein and 12 immune globulin component.

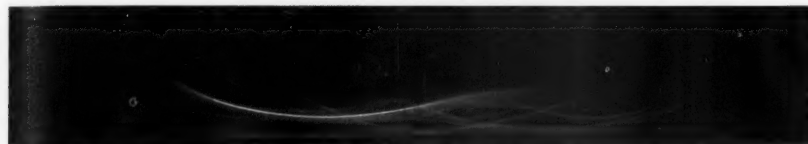


Fig. 1b. Photograph of the analysis drawn in Fig. 1a.

TABLE 1. *The heat-stability of bovine colostrum proteins as determined by immune electrophoresis.*

+ indicates presence of line; (+) indicates presence of fuzzy line.

Time min.	Temp. °C	Line number (see Fig. 1a)															
		1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16*
15	60		+	+	+	+	+	+	+	+		+	+	+	+	+	
15	65		+	+	+	+	+	+	+	+		+	+	+	+	+	
15	70		+	+	+	+		+	+	(+)		+	(+)	(+)		+	
15	75		+	+	+	+		+	(+)			+				+	
15	80		+	+	(+)			+	(+)			+				+	
15	85		+	+	(+)			+	(+)			+				+	
15	90		+	+	(+)			+	(+)			(+)				+	
15	95		+	+	(+)			+								(+)	
15	100		+	+	(+)			+								(+)	
15	120		+	+													

\* Lines 1 and 16 are seen only inconsistently in the colostrum-anti-colostrum spectra.

sera, however, gave rise to more distinct precipitates permitting more ready interpretation of these spectra. Therefore, only such immune sera were used in the present investigation.

### Method

The analyses were performed with the immune electrophoretic method of Grabar & Williams (3, 4) in the modifications described by Wadsworth & Hanson (15).

### Results

Immune electrophoretic analysis of bovine colostrum by means of anti-colostrum immune serum showed a spectrum of at least 16 precipitates. Their shape and localization are shown in Figs. 1a and 1b. Some of these precipitates have been identified with known milk and blood serum proteins (6, 8) as indicated in Fig. 1a. The heat-sensitivity of the proteins

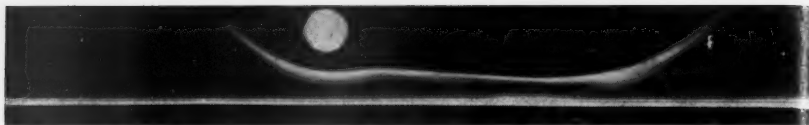


Fig. 2. Photograph of an immune electrophoretic analysis of colostrum heated to 120°C for 15 minutes. Immune serum: anti-colostrum. Only the casein precipitates (nos. 2 and 3) are seen. Anode to the right.

corresponding to the immunoprecipitates is illustrated by the results of the immune electrophoretic analyses listed in Table 1. It may be seen that exposure to heat for 15 minutes resulted in little change in the antigenic reactivity until the level of 70–75°C was employed. At this temperature the major immune globulin component among others was affected (line no. 12). Line no. 15 in the immune globulin region persisted, however, even after exposure to 100°C. The heat resistance of casein (nos. 2 and 3),  $\alpha$ -lactalbumin (no. 7) and  $\beta$ -lactoglobulin (no. 4) was notable. After treatment at 120°C only the casein precipitates were obtained (Fig. 2).

Immune electrophoretic analyses of mature milk showed a spectrum consisting of 12 precipitation lines (Fig. 3). Some of these were designated since they were identifiable with known milk proteins. Nine of them identified with precipitates in the colostrum spectrum and were given the same numbers as used in that spectrum (Fig. 1a). One precipitate was seen only inconsistently and was not included in this report, while two others in the immune globulin region could not with certainty be identified with the corresponding precipitates in the colostrum spectrum; they were therefore given separate numbers (nos. 17 and 18) in Fig. 3. All of these precipitation lines were still seen after heating the milk to 70°C for 15 minutes

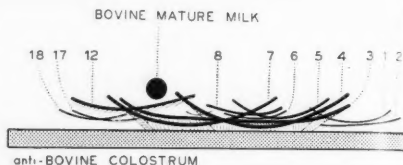


Fig. 3. Diagrammatic representation of an immune electrophoretic analysis of bovine mature milk by means of anti-colostrum immune serum. The numbers indicate the same precipitates as in the colostrum spectrum (Fig. 1a). Precipitates numbered 17 and 18 have not with certainty been identified with the corresponding precipitates in the colostrum spectrum. Anode to the right.

(Table 2); however, a temperature of 75°C changed the immune globulin component, which gave rise to the dense precipitate found in this region (no. 12). The serum albumin line in the mature milk spectrum was still apparent as a fuzzy precipitate even after exposure of the milk to 100°C. In colostrum no such precipitate was seen after heating to 75°C. With the exception of this precipitate similar results were noted for colostrum and mature milk after exposure to 100° and 120°C. As can be seen in Fig. 4, milk was also analyzed after pasteurization by different methods: heating to 64°C for 30 minutes, 75°C for 19 seconds and 90°C for 120 seconds. It was quite evident that heating to 64°C for 30 minutes caused only minor changes of the proteins while the higher temperatures had a more deleterious effect (Table 2).

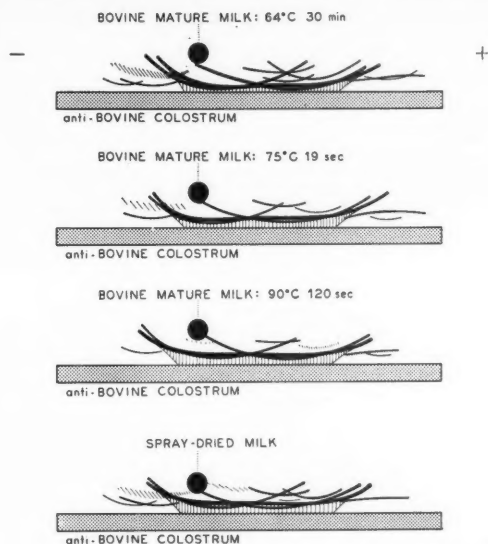


Fig. 4. Diagrammatic representations of immune electrophoretic analyses of pasteurized and spray-dried milk by means of anti-colostrum serum.

The analyses of dried milk showed that spray-drying (Fig. 4) was somewhat less harmful to the proteins than was drum-drying (Table 2). After double drum-drying the same degree of denaturation of the milk proteins was seen as for the baby-food products examined (Table 2).

The "non-allergenic" milk product for children hypersensitive to milk showed retained antigenicity of the substances forming the casein, the  $\alpha$ -lactalbumin and the  $\beta$ -lactoglobulin precipitation lines (Table 2).

### Discussion

Since the beginning of this century several investigators have studied and discussed the effect of heat on bovine milk proteins on the basis of results obtained with various immunological techniques (10, 11, 12 and others). Electropho-

retic techniques have also been applied in such studies (2, 9, 14). With the aforementioned methods it has only been possible to study a few fractions of milk or isolated proteins. By means of immune electrophoresis a descriptive and more complete picture is obtained of the changes of the milk proteins. In addition it is unnecessary to work with isolated purified substances, since each substance may be analyzed individually as a separate precipitate in the immune electrophoretic spectrum. This is also advantageous, as different heat-sensitivities have been observed for isolated milk proteins and for these same proteins in whole milk (9, 10). The actual experiments were planned to illustrate the changes detectable in processing milk. It was demonstrated that casein resisted 120°C and that  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin were still



TABLE 2. *The heat-stability of bovine mature milk and milk products as determined by immune electrophoresis.*

+ indicates presence of line; (+) indicates presence of fuzzy line.

Material	Time min.	Temp. °C	Line number (see Figs. 1 a and 3)										
			1	2	3	4	5	6	7	8	12	17	18
Mature milk	15	60	+	+	+	+	+	+	+	+	+	+	+
	15	65	+	+	+	+	+	+	+	+	+	+	+
	15	70	+	+	+	+	+	+	+	+	+	+	+
	15	75	+	+	+	+	+	+	+	+	+	+	+
	15	80	+	+	+	+	+	+	+	+	+	+	+
	15	85	+	+	+	+	(+)	+	+	+	+	+	+
	15	90	+	+	+	+	(+)	+	+	+	+	+	+
	15	95	+	+	+	+	(+)	+	+	+	+	+	+
	15	100	+	+	+	+	(+)	+	+	+	+	+	+
Pasteurized milk	30	64	+	+	+	+	+	+	+	+	(+)	+	+
	19 sec.	75	+	+	+	+	+	+	+	+	(+)	+	+
	120 sec.	90	+	+	+	+	(+)	+	+	+	+	+	+
Spray-dried milk			+	+	+	+	+	(+)	+	(+)	(+)	+	+
Single drum-dried milk			+	+	+	+	+	(+)	+	(+)	+	+	(+)
Double drum-dried milk			+	+	+	+	(+)	+	+	(+)	+	+	(+)
Baby food products:													
Semper Välling				+	+	+	(+)		+	(+)		(+)	(+)
Baby Semp				+	+	+	+		+	(+)		+	+
Allergilac				+	+	+			+				

antigenic after heating to 100°C for at least 15 minutes, whereas the major immune globulin component, as well as some other proteins, were destroyed by heating to 70–80°C for the same length of time (Tables 1 and 2). When judging the results obtained it should be noted, however, that it is not clear how the destruction of antigenicity is related to the physico-chemical changes of the proteins. Furthermore the technique employed is very sensitive and thus minimal amounts of antigenic material can be demonstrated, and visible precipitation lines may be formed by proteins present in very low quantity. Thus, if only a small amount of a substance has remained unchanged after treatment, it may still give rise to a precipitation line. It is unlikely that this con-

dition should not be observed, as in such a case the line should form further from the immune serum basin owing to the reduced quantity of antigen. Sometimes the line will appear fuzzy and out of balance since optimal proportions may not be attained. Preparations heated at somewhat lower temperatures than the one at which antigenicity of the protein was destroyed often contained the protein with such a reactivity that a fuzzy and ill-defined precipitate was formed (indicated by (+) in Tables 1 and 2).

Comparison of the results obtained with colostrum and mature milk showed that the heat-sensitivity of some proteins was different in the two fluids (cf. line 5, serum albumin, and line 6 in Tables 1 and 2). This may be explained by the afore-

mentioned difference in milieu giving rise to such differences in the heat-sensitivity (9). Several other substances showed similar reactivity. The heat-stability of casein in both colostrum and mature milk was especially notable as the casein precipitates remained quite unchanged after heating the fluids to 120° for 15 minutes.

The immune electrophoretic patterns indicated that the heat effect of the pasteurization at 64°C for 30 minutes was less drastic to the milk proteins than pasteurizations at 75°C for 19 seconds and at 90°C for 120 seconds. In the same way it was found that the proteins of spray-dried milk were somewhat less affected than those of drum-dried milk.

It was possible to analyze milk proteins in food products with the immune electrophoretic technique. These products were made of pasteurized pre-heated and spray-dried milk and the findings agreed with the changes that were obtained after heating of milk to corresponding temperatures. Immune electrophoresis may be a method of controlling the existence of milk proteins in different products and of determining the degree of denaturation of these proteins. It has recently been found that falsification of bovine blood plasma by addition of casein can easily be detected using anti-milk sera (7).

A sample of a milk product reported to be suitable for infants hypersensitive to milk ("Allergilac") was also analyzed. It was found that the  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin and casein had retained their antigenicity. These proteins have been demonstrated to be allergenic (13 and others). It may be important to realize, however, that the antigenicity of a

substance as shown with antibodies from a hyperimmunized animal is not necessarily indicative of the allergenic properties of the substance. The use of such hyperimmune sera to analyze allergens by diffusion-in-gel methods has been criticized from this point of view (1). It should be mentioned, though, that antibodies against bovine milk have been demonstrated by diffusion-in-gel methods in some sera from children hypersensitive to milk (7), and it is possible that comparative analyses of these human sera and the hyperimmune sera might give more information about the antibodies detectable in human sera and the allergenicity of bovine milk proteins.

### Summary

With immune electrophoretic analysis a descriptive and illustrative picture is obtained of the changes of the milk proteins due to heat. It is apparent that casein resists a temperature of 120°C and that  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin are still antigenic after heating to 100°C for at least 15 minutes, whereas the major immune globulin component as well as a few other proteins are destroyed after heating to 70–80°C for 15 minutes (Tables 1 and 2). The investigation concerns colostrum, mature milk, milk pasteurized by various methods, dried milk, some commercial baby food products and a sample of a milk product advertised as non-allergenic. The possible application of the reported results to the study of cow milk allergies is discussed.

### Acknowledgements

Professor Ö. Ouchterlony is thanked for current interest and advice. The skillful

technical assistance of Miss M. Wikström is appreciated as well as the linguistic help of Mrs. C. Wadsworth. The investigation was

supported by a grant to one of us (L.Å.H.) from Göteborgs Kungl. Vetenskaps- och Vitterhetssamhälle.

### References

1. AUGUSTIN, R.: Pollen antigens versus pollen allergens. Limiting aspects of gel-diffusion methods for assay purposes in allergy. *Int Arch Allergy*, 11: 218-222, 1957.
2. EWERBECK, H.: Zum Problem der Kindermilch im Lichte der modernen Milchwissenschaft. *Milchwissenschaft*, 7: 369-375, 1952.
3. GRABAR, P. and WILLIAMS, C. A.: Méthode permettant l'étude conjuguée des propriétés électrophorétiques et immuno-chimiques d'un mélange de protéines. Application au sérum sanguin. *Biochim Biophys Acta*, 10: 193-194, 1953.
4. GRABAR, P. and WILLIAMS, C. A.: Méthode immuno-électrophorétique d'analyse de mélanges de substances antigéniques. *Biochim Biophys Acta*, 17: 67-74, 1955.
5. GÜGLER, E., BEIN, M. and MURALT, G. von: Über immunoelektrophoretische Untersuchungen an Kuhmilchproteinen. *Schweiz Med Wschr*, 89: 1172-1175, 1959.
6. HANSON, L. Å.: Immunological analysis of bovine blood serum and milk. *Experientia*, 15: 471-472, 1959.
7. — Unpublished results.
8. HANSON, L. Å. and JOHANSSON, B.: Immune electrophoretic analysis of bovine milk and purified bovine milk protein fractions. *Experientia*, 15: 377-379, 1959.
9. KARTE, H.: Veränderungen der Kuhmilchproteine unter Hitzeeinwirkung. I. Physiko-chemische Veränderungen beim Pasteurisieren, Kochen und bei der Herstellung evaporierter Milch. *Z Kinderheilk*, 80: 530-559, 1958.
10. KLEINSCHMIDT, H.: Ueber Milchanaphylaxie. *Msehr Kinderheilk*, 11 Orig: 644-682, 1913.
11. MORO, E.: Biologische Beziehungen zwischen Milch und Serum. *Wien Klin Wschr*, 14: 1073-1077, 1901.
12. RATNER, B. and GRUEHL, H. L.: Anaphylactogenic properties of milk. Immunology of the purified proteins and antigenic changes resulting from heat and acidification. *Amer J Dis Child*, 49: 287-306, 1935.
13. RATNER, B., DWORETZKY, M., OGURI, S. and ASCHHEIM, L.: Studies on the allergenicity of cow's milk. I. The allergenic properties of alpha-casein, beta-lactoglobulin and alpha-lactalbumin. *Pediatrics*, 22: 449-452, 1958.
14. SCHULTE, K. E. and MÜLLER, F.: Elektrophoretische Studien an Milchproteinen. V. Die Untersuchungen von pasteurisierten Milchen und Milchprodukten mit Hilfe der Papierelektrophorese. *Milchwissenschaft*, 10: 270-274, 1955.
15. WADSWORTH, C. and HANSON, L. Å.: Comparative analysis of immune electrophoretic precipitates employing a modified immune electrophoretic technique. *Int Arch Allergy*, 17: 165-177, 1960.

Submitted Dec. 12, 1960

Accepted Dec. 19, 1960

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## ACTH-Induced Thymic Atrophy

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In roentgen examination of the thorax in children one occasionally finds either a diffuse, poorly delineated widening of the mediastinum so that the contours of the heart and big vessels cannot be definitely determined or a fairly well circumscribed tissue mass, the nature of which is sometimes obscure. Most often this mediastinal widening, whether diffuse or more distinct is composed of thymic tissue. Indeed, this mediastinal image may have such an appearance that a tumor or malformation cannot be excluded. The size and position of the thymus varies considerably in children.

In order to determine the nature of the mediastinal widening and to increase the diagnostic acumen regarding the mediastinum, one can make use of the thymolytic effect of adrenocorticosteroids. Soffer *et al.* (2) administered ACTH to 5 patients with an enlarged thymus and in all obtained a shrinkage of the thymus. Caffey & di Liberti (1) have recently reported a similar investigation. "In 7 of 8 cases in which idiopathic widening of the mediastinum was discovered by chance in random roentgenograms, the oral administration of adrenocorticosteroids caused rapid shrinkage of the mediastinal image. In

TABLE 1. *Thirteen cases of mediastinal widening treated with ACTH.*

Case no.	Age in months	Acton Prolongatun I.U		Thymolytic effect	Remarks
		daily dose	total dose		
1	6	6	18	0	Regular ACTH
2	3	10	70	+	
3	3	10	60	+	3 weeks later thymic regrowth
4	5	10	60	+	2 weeks later thymic regrowth
5	3	10	30	+	
6	8	10	70	+	
7	36	15	60	0	Ganglioneuroma
8	2	12.5	75	+	
9	1	5	45	+	
10	18	15	75	+	
11	2	10	60	+	
12	2	10	60	+	
13	2	5	40	+	

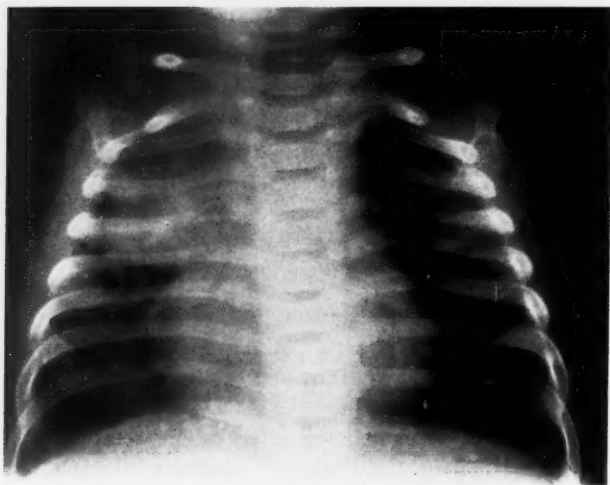


Fig. 1. Case 2. Note the tumorous mass of the upper right part of the mediastinum.

the eighth case the widened mediastinal image remained unchanged after steroid treatment."

Since 1952 we have administered ACTH to hospitalized patients with widening of the mediastinum of obscure etiology in order to achieve shrinkage of a large thymic tissue. The cases are presented in Table 1 and Figs. 1-7.

#### Results and Discussion

In 11 of the 13 cases the mediastinal widening, which was discovered by chance during roentgen examinations, decreased after the administration of ACTH. We have interpreted this shrinkage as due to atrophy of the thymus on ACTH administration. In Case 1 ACTH did not have any

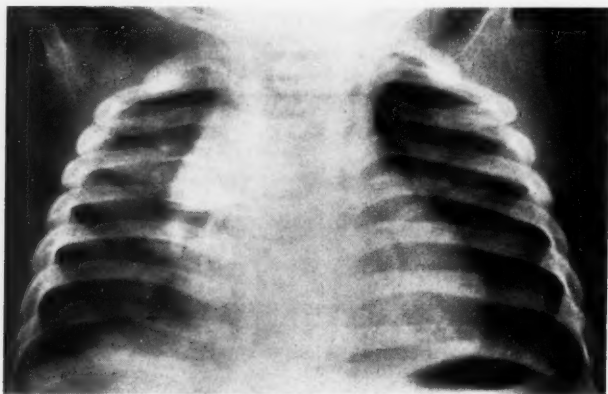


Fig. 2. Case 2. After administration of ACTH the widening shown in Fig. 1 has disappeared.

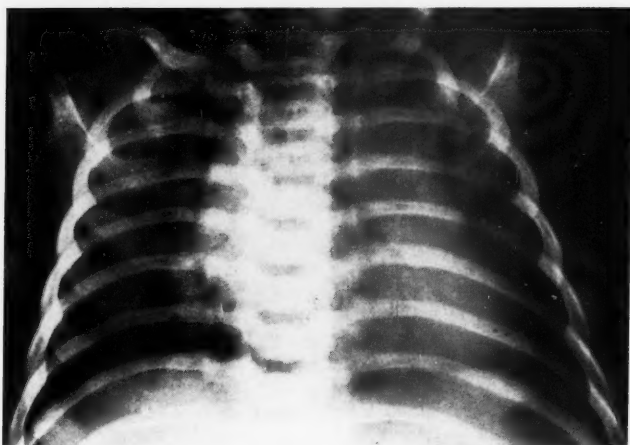


Fig. 3. Case 4. "Widening of the mediastinum" does not permit diagnosis of the heart or big vessels.

effect. The mediastinal widening can, however, have been composed of thymic tissue because the patient received, according to our present understanding, entirely too small a dose of ACTH for entirely too short a period. In Case 7 the sharply delimited formation did not decrease at all; the subsequent operation

disclosed that it was composed of a ganglioneuroma. None of the patients exhibited any undesirable reactions.

In Cases 3 and 4 renewed roentgen examination revealed that the decrease in the width of the mediastinum which was observed during ACTH administration had again increased, three and two weeks

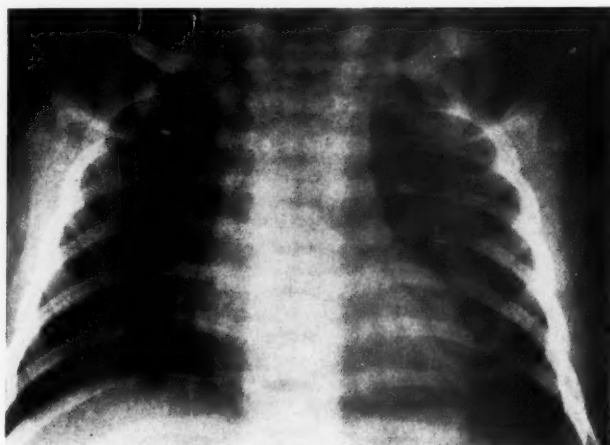


Fig. 4. Case 4. After administration of ACTH the "mediastinal widening" has disappeared.

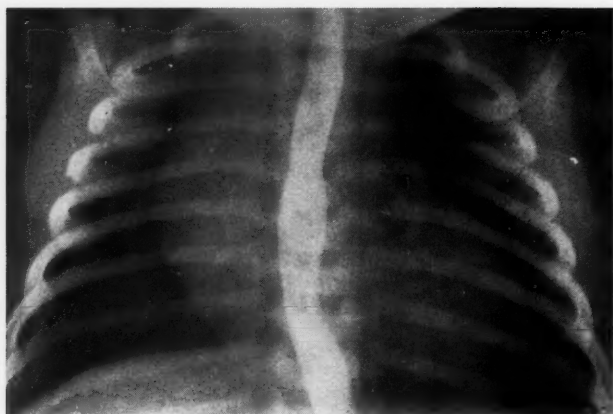


Fig. 5. Case 5. Note the "mediastinal widening."

later respectively. Thus, with the short course of ACTH therapy described above one cannot expect permanent reduction of a large thymus.

Case 7 demonstrated that administration of ACTH affords a possibility of a differential diagnosis between thymic tissue in the mediastinum and other tumorigenic tissue there. Thymic tumors, i.e. thymoma, seem to respond in the same manner as normal thymic tissue. In the

material presented by Soffer *et al.* "in 2 instances the thymic enlargement was proven to be due to tumor, 1 of which was malignant in character with proven metastases". These thymic enlargements decreased on ACTH administration.

Caffey & di Liberti's patients received adrenocorticosteroids or derivatives thereof orally (prednisone in most cases, triamcinolone in a few). The advantage of the administration of these agents over ACTH



Fig. 6. Case 5. After administration of ACTH the "widening of the mediastinum" has disappeared.



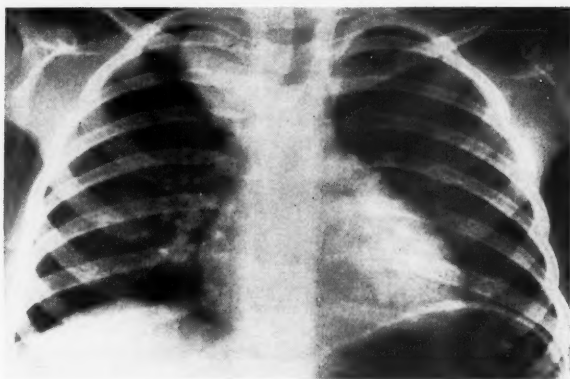


Fig. 7. A rounded tumor formation is seen apically on the right side. Administration of ACTH did not change the size or appearance of this formation. Ganglioneuroma.

is that they can be given to ambulatory patients. In Case 1 we used regular ACTH; in the others, acton prolongatum. By using the depot preparation it is possible to reduce the number of injections to one per day. The disadvantage of injection is counterbalanced by the belief that it is more physiologic to stimulate the adrenal cortex with ACTH than to depress its function with adrenocorticosteroids. However, either should be of approximately the same value as far as the desired effect on the thymic tissue is concerned.

Reduction of mediastinal widening during administration of ACTH or adrenocorticosteroids does not always necessarily indicate that it has been thymic tissue or a thymic tumor which has decreased; other tumorous masses may perhaps be affected in a similar manner by ACTH or adrenocorticosteroids. In our cases, with the exception of Case 7, there were no indications in the clinical picture or the subsequent course that the mediastinal widening should have been com-

posed of other benign or malignant tumors. In Case 1, where we evidently administered too small a dose of ACTH, the patient on examination 7 years later was healthy and roentgenography of the thorax at that time did not disclose any widening of the mediastinum.

### Summary

In 13 cases with a widening of the mediastinum, discovered in the course of roentgenologic examination, ACTH was administered to increase the possibilities of diagnosing the cause. In 11 cases there was a reduction of the width of the mediastinum. The decrease is considered to be due to the thymolytic effect of the adrenocorticosteroids. In one case the widening remained unchanged; in this case entirely too small a dose of ACTH was given. In another case the more circumscribed enlargement remained unchanged and the subsequent operation revealed that it was composed of a ganglioneuroma.

## References

1. CAFFEY, J. and DI LIBERTI, C.: Acute atrophy of the thymus induced by adrenocorticosteroids: observed roentgenographically in living human infants. *Amer J Roentgenol*, 82: 530, 1959.
2. SOFFER, L. J., GABRILOVE, J. L. and WOLF, B. S.: Effect of ACTH on thymic masses. *J Clin Endocr*, 12: 690, 1952.

Submitted Dec. 12, 1960

Accepted Jan. 16, 1961

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## Catecholamine Excretion in the Newborn Period: Effect of Short Periods of Induced Hypoxia<sup>1</sup>

by LEO STERN,<sup>2</sup> ROBERT E. GREENBERG<sup>3</sup> and JOHN LIND

The response of the newborn to induced anoxia is a many faceted one. Cross *et al.* (5, 6) reported that a reduction in the oxygen content in the inspired air from 20 % to 15 % caused a fall in O<sub>2</sub> consumption in newborn term and premature infants. Cross and co-workers have further shown that, in response to the administration of 15 % O<sub>2</sub>, premature and full term infants show only a transitory hyperventilatory response which returns to its initial level after approximately two minutes (7). Rowe & James produced changes in pulmonary arterial pressure as well as a fall in the arterial oxygen saturation in a newborn infant by the administration of a gas mixture containing 15 % oxygen, though the effects were more striking when the oxygen content was lowered to 10 % (21).

Asphyxia, produced by tracheal compression, has been found to result in intense activation of the adrenal medulla in animals. Rapela & Houssay observed a striking increase in the secretion of the adrenal medulla of the dog in asphyxia (20),

similar findings were recorded in the cat by Euler & Folkow (12). In both studies the percentage of noradrenaline in the supra-renal venous blood was similar to the resting state.

It has also been suggested by Dawes and co-workers that the release of catecholamines in response to asphyxia provides an alternative method of closure of the ductus arteriosus in newborn lambs (9). (Paradoxically the other stimulus to closure is an increase in oxygen tension.) Clinically, Englesson, Rooth & Sjöstedt have recently proposed the routine use of 15 % oxygen as a milieu for premature infants on the supposition that such a mixture is more suited physiologically to their metabolic requirements (11).

The following study was undertaken in order to evaluate the possible relationships between the administration of a reduced oxygen mixture and the excretion of noradrenaline and adrenaline in the newborn infant. Coincident with the above, the study afforded an opportunity to assess normal dopamine excretion in the newborn period.

### Methods

Twenty-one full term male infants, ranging in age from 1 to 18 days, were studied.

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The infants were adjudged mature by birth weight ( $> 2500$  g) and/or clinical appearance. At the time of study they ranged in weight from 2300 g to 4480 g. In addition, the study was carried out on four premature infants weighing between 1690 and 2070 g.

Urine was collected according to previously described methods (18). Immediately following collection of the control specimen the infant was placed in a special hood used clinically for purposes of antibiotic inhalation therapy. The inlet was attached to a tank containing a commercially prepared mixture of 84.5% nitrogen and 15.5% oxygen (AGA); the infant was exposed to this mixture for 15 minutes. The oxygen concentration, in front of the infant's airway, was repeatedly checked during the period of administration with a Beckman  $D_2$  oxygen analyzer, accurately adjusted to read 100% in pure oxygen and 20.9% in room air. An oxygen concentration of less than 16% was reached within 1 minute after the administration of the gas mixture was begun in all infants studied. Respiratory rates were recorded before, during and at the conclusion of the test period. The next observed voiding was collected and the time noted. Two to three drops of 6N HCl were added to the urine specimens and they were immediately frozen; analysis for catecholamines was done usually within 24 hours.

Urinary noradrenaline and adrenaline were determined according to the method of Euler & Lishajko (13). Fluorescence was read in a Coleman photofluorometer, Model 12C, with appropriate filters. Dopamine was determined according to the fluorimetric method of Carlsson & Waldeck (2), after adsorption on an alumina column and elution with 0.25 N acetic acid. An Aminco-Bowman spectrophotofluorometer was used for these determinations. It was confirmed that noradrenaline, adrenaline, normetanephrine, metanephrine and 3,4-dihydroxyphenyl-acetic acid (dopac) did not produce significant fluorescence under the conditions of the method used. All values were calculated and expressed as nanog per kg body weight per minute (1 nanog = 0.001 microg).

## Results

### *The Effect of a Reduced Oxygen Atmosphere on the Excretion of Noradrenaline and Adrenaline*

Clinically, no adverse effects were noted as a result of the administration of the gas mixture used. Respiratory rates remained unchanged in all infants. Rectal temperatures in the full term infants ranged from 35.9 to 37°C. The results for noradrenaline excretion are shown diagrammatically in Fig. 1 and for adrenaline excretion in Fig. 2. The mean values for noradrenaline excretion pre- and post-stimulus are  $0.301 \pm 0.188$  and  $0.340 \pm 0.177$  nanog/kg/minute, respectively. Similar values for adrenaline excretion are  $0.056 \pm 0.042$  and  $0.085 \pm 0.054$  nanog/kg/minute, respectively.

From the above, it is apparent that there is no significant difference between the pre- and poststimulus levels for either noradrenaline or adrenaline when the infants are evaluated as a group. Age had no apparent influence on adrenaline excretion. However, whereas only 1 of 10 infants under 5 days of age showed an increase in noradrenaline excretion following exposure to the gas mixture, 7 of 11 infants over 5 days of age demonstrated an increase of 20% or more in the poststimulus period. Fig. 3 shows this relationship between increasing age and the cumulative percentage of infants who responded to the stimulus with an increase in urinary excretion for both noradrenaline and adrenaline. None of the four prematures studied showed an increase in either noradrenaline or adrenaline (Fig. 4).

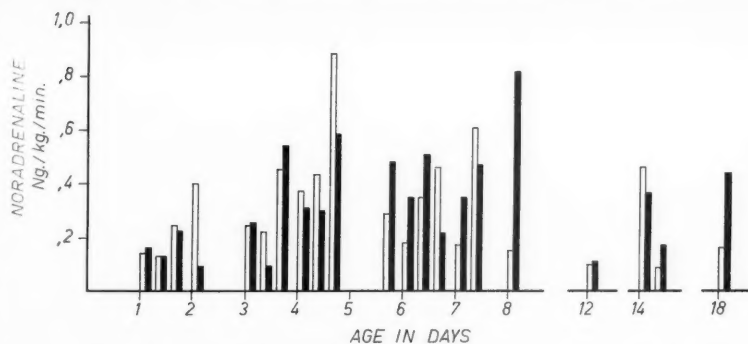


Fig. 1. Urinary excretion of noradrenaline, in response to 15.5 %  $O_2$ , at different postnatal ages. The clear blocks indicate "pre-stimulus" levels, while the dark blocks indicate the corresponding "poststimulus" values. Expressed as nanog excreted/kg body weight/minute.

#### *The Excretion of Dopamine in the Newborn Period*

No positive correlation between dopamine excretion and the hypoxic stimulus was found. To assess the mean values, then, for dopamine excretion, all values obtained for both dopamine and noradrenaline on each newborn studied were averaged, with the results shown in Table

1. The mean excretion of dopamine is  $2.96 \pm 1.59$  nanog/kg/minute as compared to  $0.314 \pm 0.123$  nanog/kg/minute for noradrenaline. Dopamine, then, is excreted in amounts approximately 9 times that of noradrenaline. Fig. 5 shows that the level of urinary dopamine increases in a rough correlation with increasing levels of urinary noradrenaline. Statistically significant dif-

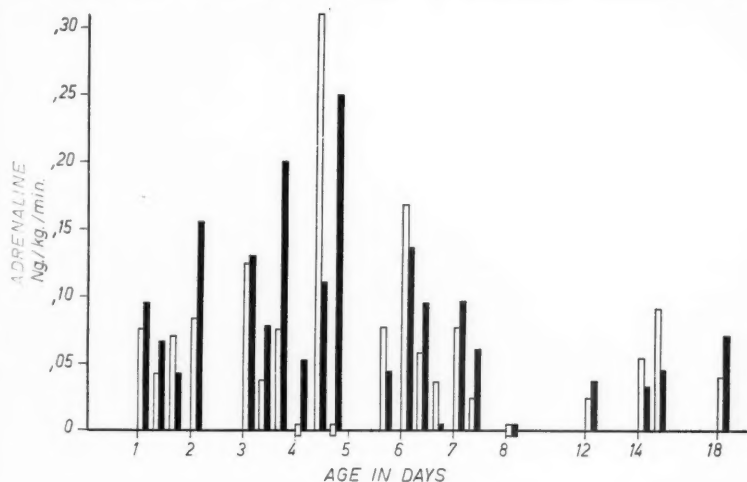


Fig. 2. Urinary excretion of adrenaline, in response to 15.5 %  $O_2$ , at different postnatal ages. Legend as for Fig. 1.

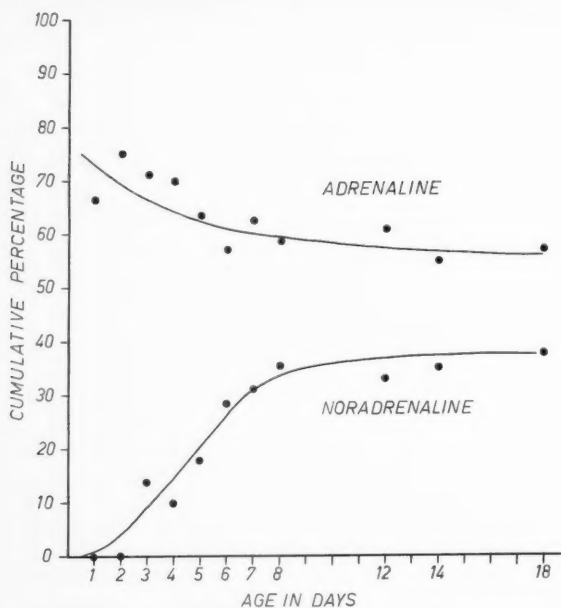


Fig. 3. Relation between cumulative percentage (aggregated percentage of the total group) showing a positive response to the stimulus and age. Note that whereas the curve for adrenaline slopes only slightly, that for noradrenaline rises in an S-shape, levelling off with increasing age.

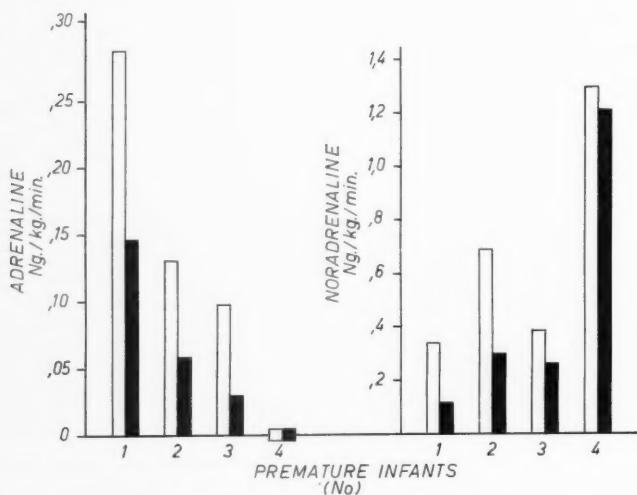


Fig. 4. "Pre-" and "post-stimulus" levels of adrenaline and noradrenaline in the urine of four premature infants. Legend as for Fig. 1.

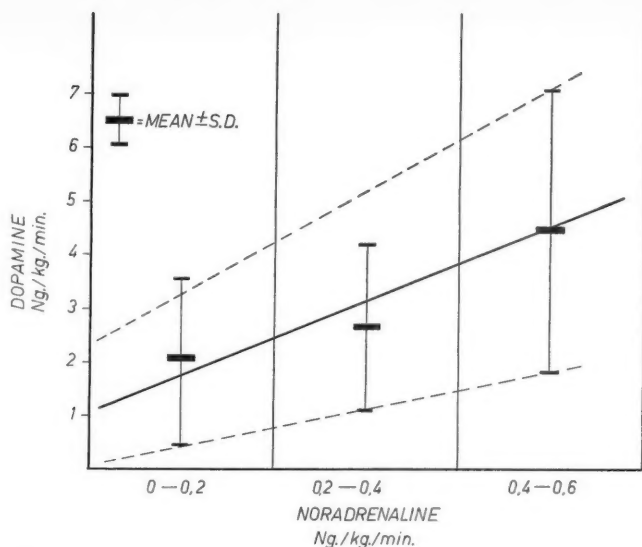


Fig. 5. The relation between dopamine and noradrenaline excretion in the newborn period.

ferences in dopamine excretion, when compared to levels of noradrenaline, are not present, due to the wide range of dopamine values.

### Discussion

#### *The Response to a Reduced Oxygen Atmosphere*

The failure of this stimulus to result in any obvious clinical effects, and the apparent readiness with which the infants tolerated it has been noted by Cross (8); similarly, no changes in respiratory or heart rates or in general clinical condition were found in the group of prematures given 15% oxygen by Englesson, Rooth & Sjöstedt (11). Thus, it would seem that this degree of hypoxia is without apparent adverse effect on premature and full term infants.

Many stimuli are known to evoke ca-

techolamine release in the adult human or animal. It has been previously demonstrated, using the methods employed in this study, that newborn infants are capable of responding to change of posture and insulin hypoglycemia, for example, with an increase in catecholamine excretion (18).

Animal experiments have demonstrated that asphyxia can induce a reflex activation of the adrenal medulla (12, 20). Although it has not been quantitatively assessed, it would appear that the degree of anoxia must be severe before the adrenal response is significant. As the peripheral sympathetic nervous system also participates in the asphyxial defense reaction (4); such activation should effect changes in urinary noradrenaline, to which both the peripheral sympathetic nervous tissue and the adrenal medulla contribute.



TABLE 1. *The urinary excretion of dopamine (hydroxytyramine) as compared to noradrenaline in normal newborn infants.*

Age days	Weight kg	Noradrenaline ng/kg/min	Dopamine
1.1	3.17	.152	1.79
1.5	3.10	.131	1.12
1.7	3.19	.235	3.42
1.9	3.20	.242	1.97
3.3	4.48	.158	3.11
3.4	4.17	.323	2.46
3.9	3.48	.508	2.94
4	3.60	.343	2.91
6	2.51	.418	1.99
6	2.72	.435	3.16
6	3.82	.324	5.88
7	3.38	.331	3.41
8	3.48	.492	1.69
14	2.63	.225	1.54
18	3.60	.402	6.95
Mean =		.314	2.96
S.D. =		±.123	± 1.59

Since, in this study, the mean pre- and post-stimulus values for both noradrenaline and adrenaline are not significantly different, when analyzed as an entire group, it would appear that either the newborn infant is incapable of responding to this stimulus with an increase in catecholamine excretion or the stimulus itself is not of sufficient severity to produce such a response. In either case, this would suggest that physiologic responses reported as a result of the administration of hypoxic mixtures of this degree are mediated by mechanisms other than catecholamine release.

A more detailed analysis showed that age did not affect the randomness of the response in terms of adrenaline excretion. However, whereas 7 of 11 infants over 5 days of age exhibited an increase in noradrenaline excretion of at least 20% after exposure to the gas mixture, only 1 of 10

infants under 5 days of age demonstrated such a response. If this finding is valid, it would suggest that either there is a delay in the maturation of the central response to hypoxia (with respect to noradrenaline release) or that the response to a similar degree of hypoxia is qualitatively different with increasing postnatal age.

#### *The Urinary Excretion of Dopamine*

Dopamine was originally demonstrated to be a substance normally found in human urine by Holtz *et al.* (19). Euler, Hamberg & Hellner confirmed its presence in human urine by chromatographic techniques (14). Drujan *et al.* (10) found dopamine in the urine of adults in levels of  $198 \pm 37$  microg per day, approximately 5-10 times that of noradrenaline. From our studies, it is apparent that a similar dopamine:noradrenaline ratio is present in the urine of newborn infants.

The physiologic significance of dopamine in tissues or urine has yet to be established. It has been demonstrated that dopamine serves as a precursor in the biosynthesis of both noradrenaline and adrenaline in the adrenal gland (13), and of noradrenaline in sympathetic nervous tissue (17). Of the entire sequence of reactions in the formation of adrenaline from phenylalanine, the enzyme system that catalyzes the hydroxylation of dopamine to noradrenaline is least understood. Further, Senoh *et al.* (22) have recently shown that dopamine can form 2, 4, 5-trihydroxyphenethylamine (hydroxycopamine) both *in vivo* and *in vitro*, a compound extremely difficult to separate from noradrenaline by chromatographic techniques; they note that previous studies on

cerning the biosynthesis of noradrenaline from dopamine must be re-evaluated in the light of these findings. If, as is likely, noradrenaline is indeed formed by the hydroxylation of dopamine, recent studies suggest that such a precursor role is not the only function of dopamine. In brain, dopamine is present in high concentrations in the caudate nucleus, whereas noradrenaline is most abundant in the hypothalamus (3). Further, in ruminants, a high concentration of dopamine is found in many tissues, including lung, duodenum and liver, unrelated to other catecholamines; the dopamine content varies with the numbers of a special type of chromaffin cell (1). These studies would suggest that dopamine has a physiologic role of its own in those areas where it is present in high concentration. Dopamine has not, however, been detected in either bovine or human plasma under normal conditions, in spite of its presence in both tissues and urine (15, 23). It would thus seem that the kidney itself contributes significantly to the relatively high urinary dopamine levels; as such, the urinary excretion of dopamine can probably not be used as an index of endogenous extra-renal production. The delineation of the functions of dopamine in the organism remains as an important and interesting task.

### Summary

It has previously been demonstrated that newborn infants are capable of responding to change of posture and insulin hypoglycemia with an increase in catecholamine excretion. Similarly, pre-

vious studies by other workers have shown that severe anoxia, at least, evokes a marked release of catecholamines in animals. When newborn infants were exposed for 15 minutes to a gas mixture containing 15.5% oxygen and 84.5% nitrogen, no significant change in the urinary excretion of noradrenaline and adrenaline ensued. This would suggest that either the newborn is incapable of responding to this stimulus with an increase in catecholamine excretion or the stimulus itself is not of sufficient severity to produce such a response. In either case, any physiologic response in newborns following exposure to a hypoxic mixture of this degree, would then be mediated by mechanisms other than catecholamine release.

The study also suggests, however, that increasing postnatal age exhibits a positive correlation with the response to the hypoxic stimulus used, in terms of increases in noradrenaline excretion. The validity of these findings as well as their significance must await confirmatory studies.

Dopamine was found to be present in the urine of newborn infants in amounts roughly nine times that of noradrenaline, a ratio parallel to that observed by others for adults. No correlation between the hypoxic stimulus and dopamine excretion was found. The present confusion concerning the significance of dopamine, in both urine and tissues, is briefly reviewed.

### Acknowledgements

We would like to express our thanks to Miss Birgitta Gauffin and the nursing staff at Södra Barnbördshuset for their invaluable assistance.

## References

1. BERTLER, A., FALCK, B., HILLARP, N.-Å., ROSENGREN, E. and TORP, A.: Dopamine and chromaffin cells. *Acta Physiol Scand*, 47: 251, 1951.
2. CARLSSON, A. and WALDECK, B.: A fluorimetric method for the determination of dopamine (3-hydroxytyramine). *Acta Physiol Scand*, 44: 293, 1958.
3. CARLSSON, A.: The occurrence, distribution and physiological role of catecholamines in the nervous system. *Pharm Rev*, 11 (Pt. 2): 374, 1959.
4. CELANDER, O.: The range of control exercised by the "sympathic-adrenal system". *Acta Physiol Scand*, 45: Suppl. 116, 1954.
5. CROSS, K. W., TIZARD, J. P. M. and TRYTHALL, D. A. H.: The metabolism of newborn infants breathing 15% oxygen. *J Physiol*, 129: 69, 1955.
6. — Gaseous metabolism of the newborn infant breathing 15% oxygen. *Acta Paediatr*, 47: 217, 1958.
7. CROSS, K. W.: Factors affecting O<sub>2</sub> consumption in newborn infants. In *Adaptation to Extrauterine Life*, ed. T. K. Oliver, Ross Lab., Columbus, 1960.
8. — Personal communication.
9. DAWES, G. S.: Changes in the circulation at birth and the effects of asphyxia. Recent *Advances in Paediatrics*, ed. D. Gairdner, T. and A. Churchill Ltd., London, 1958.
10. DRUJAN, B. D., SOURKES, T. L., LAYNE, D. S. and MURPHY, G. F.: The differential determination of catecholamines in urine. *Canad J Biochem Physiol*, 37: 1153, 1959.
11. ENGLESSON, G., ROTH, G. and SJÖSTEDT, S.: Treatment of premature infants with 15% oxygen. *Acta Paediatr*, 48: Suppl. 118: 42, 1959.
12. EULER, U. S. v. and FOLKOW, B.: Einfluss verschiedener afferenter Nervenreize auf die Zusammensetzung des Nebennierenmarkinkretes bei der Katze. *Arch Exp Path & Pharm*, 219: 242, 1953.
13. EULER, U. S. v. and LISHAJKO, F.: The estimation of catecholamines in urine. *Acta Physiol Scand*, 45: 122, 1959.
14. EULER, U. S. v., HAMBERG, U. and HELLNER, S.:  $\beta$ -(3:4-dihydroxyphenyl) ethylamine (hydroxytyramine) in normal human urine. *Biochem J*, 49: 655, 1951.
15. EULER, U. S. v., FLÖDING, I. and LISHAJKO, F.: The presence of free and conjugated 3:4-dihydroxyphenylacetic acid (Dopa) in urine and blood plasma. *Acta Soc Med Upsal*, 64: 217, 1959.
16. GOODALL, McC. and KIRSHNER, N.: Biosynthesis of adrenaline and noradrenaline in vitro. *J Biol Chem*, 226: 213, 1957.
17. — Biosynthesis of epinephrine and norepinephrine by sympathetic nerves and ganglia. *Circulation*, 17: 366, 1958.
18. GREENBERG, R. E., LIND, J. and EULER, U. S. v.: Effect of posture and insulin hypoglycemia on catecholamine excretion in the newborn. *Acta Paediatr*, 49: 780, 1960.
19. HOLTZ, P., CREDNER, K. and KOEPP, W.: Die enzymatische Entstehung von Oxytyramine in Organismus und die physiologische Bedeutung der Dopadecarboxylase. *Arch Exp Path & Pharm*, 200: 356, 1942.
20. RAPELA, C. E. and HOUSSAY, B. A.: Adrenalina y noradrenalina de la sangre suprarrenal del perro durante la asfisia. *Rev Soc Argent Biol*, 28: 7, 1952.
21. ROWE, R. D. and JAMES, L. S.: Pattern of response of pulmonary and systemic arterial pressures in newborns and older infants to short periods of hypoxia. *J Pediatr*, 51: 7, 1957.
22. SENOH, S., CREVELING, C. R., UDENFRIEND, S. and WITKOP, B.: Chemical, enzymatic and metabolic studies on the mechanism of oxidation of dopamine. *J Amer Chem Soc*, 81: 6236, 1959.
23. WEIL-MALHERBE, H. and BONE, A. D.: The fluorimetric estimation of adrenaline and noradrenaline in plasma. *Biochem J*, 67: 65, 1957.

Submitted Aug. 20, 1960

Accepted Jan. 13, 1961

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## The "Mercury-In-Rubber" Strain Gauge for Measurements of Blood Pressure and Peripheral Circulation in Newborn Infants

by OLOV CELANDER and GÖRAN THUNELL

In previous communications (1, 2) it was described how venous occlusion plethysmography could be successfully performed on newborn infants for measuring changes in leg blood flow, peripheral resistance to flow and systemic blood pressures. In a further study (3) on medical students the plethysmographic method for blood pressure determinations was found to agree satisfactorily with simultaneous intraarterial pressure recordings from the corresponding artery. However, in clinical work the use of a water-filled plethysmograph and the recording on an ordinary kymograph on smoked paper is rather inconvenient. In this paper will be described how these difficulties might be overcome by the use of the "mercury-in-rubber" gauge which was introduced in the study of peripheral circulation in man some years ago by Whitney (5).

### Methods

#### Principle

The "mercury-in-rubber" gauge operates as a fairly distensible electrical strain gauge. A suitable length of thin and highly distensible rubber tubing is filled with mercury and fitted to the extremity. The electrical

resistance of the mercury column is dependent on the circumference of the leg and, therefore, on leg volume. An increase of leg volume will distend the gauge and, as the mass of mercury remains constant, the length of the mercury column will increase on behalf of a corresponding decrease of thickness. Both these changes will produce an increase of electrical resistance. The gauge is part of a balanced Wheatstone bridge, fed with a low-voltage, high-frequency current. The bridge, being balanced for a particular degree of extension of the tube, will allow changes of extension (plus or minus) to be recorded by the current flowing in the bridge galvanometer circuit. Whitney (5) has shown that "a good linear relationship exists between the bridge output recorded by the galvanometer and the percentage extension or contraction of the gauge". To attain maximal sensitivity of the "mercury-in-rubber" gauge we have (following the recommendations to us by Dr C. J. Eagan) made the gauge make up practically the entire resistance of one arm of the bridge. The current feeding the bridge was obtained from an ordinary electromanometer (Elema). The current flowing in the galvanometer circuit of the bridge was amplified in the electromanometer and could be further amplified by the amplifier in the electrocardiograph (Elema Cardirex 3B), which was used for the recording. These general principles are illustrated in a simplified form in Fig. 1.

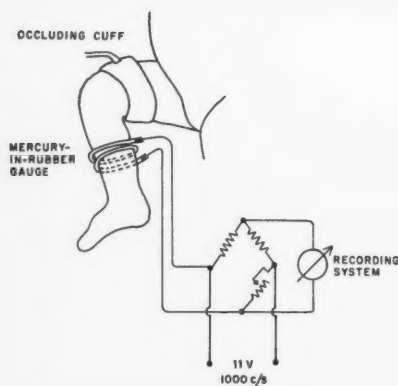


Fig. 1. A schematic drawing of the "mercury-in-rubber" strain gauge and the Wheatstone bridge.

#### *The "mercury-in-rubber" gauge*

The rubber tubing used has the transverse dimensions of 1.1 mm (outer diameter) and 0.35 mm (inner diameter).<sup>1</sup> The leads to the bridge are fine insulated multi-strand copper wire. The contacts which intrude into the rubber tube have been made of 0.7 mm silver threads, 4 cm of length, soldered up in one end with the copper wire. The other end, which is to be introduced into the tubing, is soldered up with two thinner (0.18 mm) silver threads; to facilitate contact with the mercury they protrude about 1 cm beyond the tip of the thicker silver thread.

The filling of the tube with mercury requires pains and practice. It was found convenient to place the tube level on a firm table. An ordinary 5 cc glass syringe supplied with a thin hypodermic needle, which has a dulled tip, was found suitable. The prepared tips of the silver leads were then carefully introduced and the tube ends were sealed by tight ligatures. Some "over-filling" with mercury was found desirable. The gauge was now tested for electrical continuity and for its capacity to withstand rather violent handling.

<sup>1</sup> Latex rubber tubing (nr 27), obtained from Huntington Rubber Mills, Box 570, Portland, Oregon, U.S.A.

#### *The transformer coupling device*

The circuit diagram is shown in Fig. 3. The bridge excitation ( $A-B$ ) is supplied by the ordinary Elema electromanometer, which normally supplies a pressure transducer with an alternating current of 11 V at 1000 cps.  $T_1$  and  $T_2$  are matching transformers.  $R$  is a variable resistance chosen to match the resistance of the "mercury-in-rubber" gauge ( $1-2$ ). The current over the bridge ( $C-D$ ) supplies the electromanometer and is recorded on the electrocardiograph. After balancing the bridge for the particular extension of the rubber tube, when fitted to the calf of the child, a suitable amplification is chosen on the electromanometer and the electrocardiograph.

#### *The use of the "mercury-in-rubber" gauge on newborns*

The general principles for plethysmography and the use of this method for blood-pressure determinations have been described elsewhere (1, 2). To be successful in using the highly sensitive "mercury-in-rubber" gauge, mechanical disturbances have to be minimal. The child, therefore, has to be at complete rest, preferably asleep during the measurements. The blood-pressure cuff, the mercury manometer and the pressure reservoir are the same as those previously described by Celander & Thunell (2). Fig. 2 shows the apparatus and the gauge fitted to the calf of a newborn.

#### **Results**

Fig. 4 shows, at high amplification, the minute volume changes in the leg of a newborn which accompany the arterial pulse waves. From this tracing it will be easily realized that the electrical strain gauge is well suited as a "pulse indicator", when measurement of the systolic arterial blood pressure is desired. As a matter of fact, the amplification of the arterial

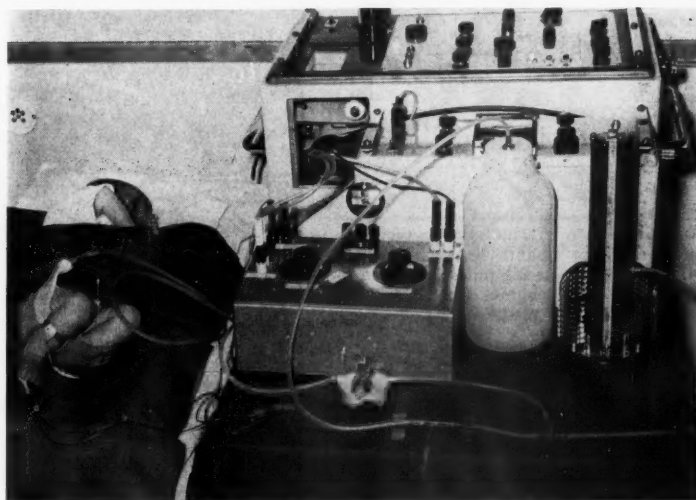


Fig. 2. The "mercury-in-rubber" gauge fitted to the calf of a newborn infant. The pressure reservoir for the cuff, the mercury manometer and the box containing the bridge are shown in front of the electromanometer and the electrocardiograph.

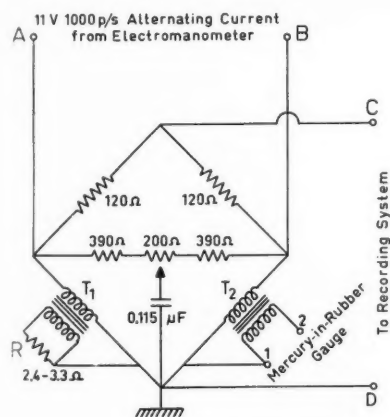


Fig. 3.

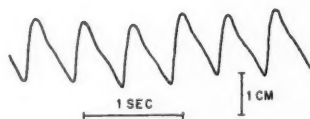


Fig. 4.

Fig. 3. Circuit diagram for the transformer coupling device. An Elema electromanometer supplies the 11 volt, 1000 cycles per second bridge excitation at *A* and *B*. The bridge output, connected to *C* and *D* is amplified by the electromanometer and the recording electrocardiograph. The "mercury-in-rubber" gauge is connected to points 1 and 2.

Fig. 4. Arterial pulsations in the leg of a newborn recorded by the "mercury-in-rubber" gauge. High amplification. The tracing illustrates the possibilities of the electrical strain gauge as a pulse indicator for measuring systolic blood pressure.



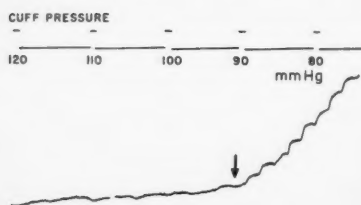


Fig. 5. A determination of the systolic blood pressure in a newborn infant. Note the appearance of prominent arterial pulsations and the sudden increase of leg volume once the cuff pressure is released to pressures below 90 mm Hg.

pulsations is practically unlimited, although in ordinary use a considerably lesser degree of amplification is quite sufficient for this purpose. How such a determination of systolic pressure can be achieved is illustrated in Fig 5. The occluding cuff fitted to the thigh was first inflated at a suprasystolic pressure which was then released at a constant speed, marked in the upper tracing. The tracings allow an accurate determination of the systolic pressure in the artery to the extremity for two reasons. Firstly, the appearance of arterial pulsations beyond the occluding cuff at a pressure of 90 mm Hg is good evidence that at this very level the intraarterial systolic peaks superseded the cuff pressure. Previous rhythmic variations in the bottom tracing were due to the respiration of the child and were not synchronous with the heart rate. Secondly, at the same cuff pressure, the tracing of the "mercury-in-rubber" gauge suddenly rises rather abruptly. This sudden increase of leg volume reflects the rushing of blood into the extremity once the cuff pressure is no longer effective in interrupting arterial inflow but still high enough to block the venous outflow from the extremity. In this child the two phenomena

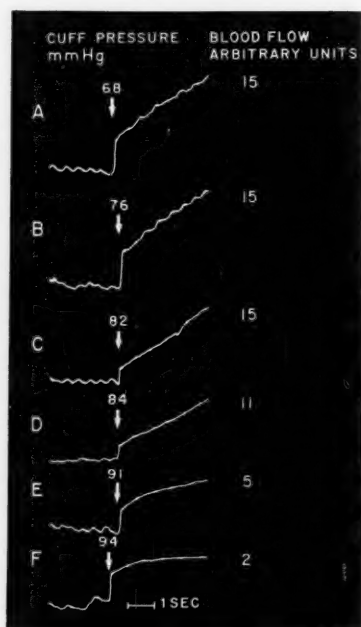


Fig. 6. Measurements of the rate of arterial inflow in relative units at various cuff pressures during the collecting periods. The systolic pressure must be very close to 94 mm Hg (which is the highest cuff pressure at which a minimal arterial inflow was recorded). Similarly, diastolic pressure must be very near 82 mm Hg (the highest cuff pressure for maximal arterial inflow). Note at C, D, E and F that only minimal arterial pulsations remain in the tracings in spite of clearly positive flow records.

coincided at a cuff pressure of 90 mm Hg. In others, as will be seen, they did not. Under such circumstances the determination of systolic blood pressure was decided on the criterion of an actual arterial inflow rather than the less reliable presence of arterial pulsations.

Fig. 6 and Fig. 7 illustrate how the "mercury-in-rubber" gauge can be used to measure both systolic and diastolic blood pressure on the basis of blood-flow determinations at various cuff pressures, as was recently suggested by Celander &



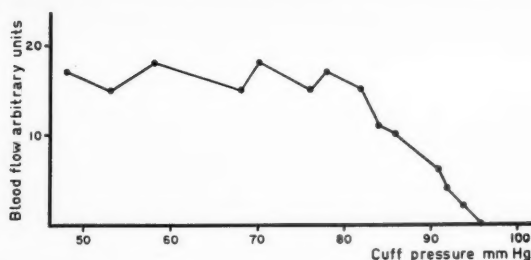


Fig. 7. Diagram showing the relation between the pressure in the cuff and the rate of arterial inflow (relative units) to the extremity.

Thunell (2). In the experiment referred to in Fig. 6 the highest cuff pressure at which a minimal arterial inflow could be recorded was 94 mm Hg, whereas the highest cuff pressure giving a maximal rate of arterial inflow was 82 mm Hg. These two values are representative of systolic and diastolic blood pressure respectively. In the diagram in Fig. 7 the total number of observations on blood flow at this occasion is plotted against cuff pressure. From this diagram it is apparent that both systolic and diastolic blood pressure can be determined with a high degree of accuracy by this approach.

### Discussion

The "mercury-in-rubber" strain gauge was originally suggested by Glaser in 1939 (4) to study respiratory moments. After it was adopted by Whitney in 1953 (6) in the study of peripheral circulation in man it has been successfully used in a great number of circulatory studies, when ordinary plethysmographs are difficult to handle. In this study the "mercury-in-rubber" gauge has been used to determine with great precision at which cuff pressure arterial pulse waves pass beyond the oc-

cluding blood pressure cuff. The practically unlimited possibilities of amplification give the electrical strain gauge advantage as a pulse indicator when arterial pulsations are small as is the case in newborns and in patients with occlusive arterial disease. Of probably greater importance is the fact that the electrical strain gauge also clearly shows at which pressure (upon gradual lowering of the cuff pressure) an arterial inflow to the extremity starts. The fact that arterial pulsations might be absent or small in spite of positive flow records (see, e.g., Fig. 6) has previously been commented upon (2) and gives further status to the electrical strain gauge plethysmograph when compared with other sphygmomanometric methods.

Venous occlusion plethysmography of the foot and calf can be used on newborns for measuring not only the systolic but also the diastolic systemic blood pressure (2). This new principle for indirect blood pressure determinations has been checked against the intraarterial blood pressure in adults (3). The "mercury-in-rubber" gauge has not yet been similarly compared with direct intravascular pressure recordings, but there seems to be no reason why the

electrical strain gauge plethysmograph should differ in these respects from the ordinary water-filled plethysmograph.

In taking blood-pressure readings, based on plethysmography, it is not necessary to measure the rate of blood flow in quantitative terms. It is sufficient to determine the highest cuff pressures at which minimal and maximal arterial inflow is obtained. However, in one and the same individual the "mercury-in-rubber" gauge might be of value for following relative changes in the rate of blood flow during a restricted period of time, such as, e.g. during the first few hours of life, or during experimentally induced alterations in the environment of the child, such as, e.g. changes in oxygen tension or temperature. In situations when the rate of blood flow is desired in quantitative terms the ordinary water-filled venous

occlusion plethysmograph should probably be preferred. A calibration of the "mercury-in-rubber" gauge can be achieved but is difficult and may be unreliable for any longer period of time.

### Summary

A modification of the "mercury-in-rubber" strain gauge is described which is suitable for clinical studies on infants. The gauge can either be used as a pulse-indicator for measuring the systolic blood pressure, or it can be used for plethysmography to study blood flow and resistance to flow in relative terms as well as systolic and diastolic arterial blood pressure.

### Acknowledgement

We wish to thank Mr H. Sundström, Elema-Schönander, Gothenburg, for his valuable suggestions and technical assistance.

### References

1. CELANDER, O.: Blood flow in the foot and calf of the newborn. *Acta Pædiat (Ups)*, 49: 488, 1960.
2. CELANDER, O. and THUNELL, G.: A plethysmographic method for measuring the systolic and diastolic blood pressure in newborn infants. *Acta Pædiat (Ups)*, 49: 497, 1960.
3. CELANDER, O. and THORÉN, O.: A comparison between a plethysmographic method for measuring the systolic and diastolic blood pressure and intraarterial pressure from the corresponding artery. To be published.
4. GLASER, V.: Eine Apparatur zur elektro-optischen Registrierung der Atembewegungen. *Elektro-pneumograph. Klin Wschr*, 18: 1156-1157, 1939.
5. WHITNEY, R. J.: The measurement of volume changes in human limbs. *J Physiol (Lond)*, 121: 1-27, 1953.
6. WHITNEY, R. J.: The electrical strain gauge method for measurement of peripheral circulation in man. In *Peripheral Circulation in Man*, ed. G. E. W. WOLSTENHOLME, A Ciba Found. Symposium, Churchill, London 1954.

Submitted Dec. 15, 1960

Accepted Dec. 19, 1960

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## CASE REPORT

# A Case of Retro-Pharyngeal Lymphosarcoma Presenting with Blindness

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The sudden onset of blindness is fortunately very rare in childhood. We report the case of a three-year-old boy who presented in this way and who was subsequently proved to have a lymphosarcoma originating in the nasopharynx.

### Case

C.F., a boy of 4½, was seen because one month earlier he had suddenly become blind. He was the second child of healthy parents and had made normal progress both mentally and physically up to the onset of this illness. Five weeks before admission he fell off a swing but did not appear to suffer any ill-effects at the time. He continued to play normally throughout the day, although he complained once or twice of pain in the region of the right eye. During the next week he again had some pain in the right frontal region and was awakened on two occasions because of headache. On Sept. 3, 1955, a week after the fall, his mother noticed that his eyes seemed abnormal. He rolled them from side to side and stared ahead without appearing to focus on anything. His mother suspected that he was blind and this was confirmed at his local hospital.

He was admitted to the West End Hospital for Nervous Diseases where he was found to be a rather quiet, apathetic child in whom the abnormal physical signs were confined to the central nervous system. In addition to his blindness, he was found to

be generally hypotonic and his knee and ankle jerks were unobtainable in both legs. Investigations at that time included X-rays of skull and chest, which were both normal, haemoglobin 15.4 g%, W.B.C. 12,000/mm<sup>3</sup> (neutrophils 47%, lymphocytes 50%, monocytes 2%, basophils 1%) and B.S.R. 9 mm in 1 hour (Westergren). The C.S.F. contained 1 cell/mm<sup>3</sup> and 25 mg% protein. The probable diagnosis was thought to be either an acute bilateral retro-bulbar neuritis or bilateral spasm of the central retinal arteries. Treatment with cortisone (12.5 mg twice daily) was begun. There was no return of vision and a complete external ophthalmoplegia developed on the right. Three weeks after the onset of the blindness, it was noticed that the cervical lymph nodes were enlarged and that there was some degree of proptosis, more noticeable on the left than the right. In addition he was becoming drowsy and increasingly apathetic, so in view of the deterioration in his general condition he was transferred to the Hospital for Sick Children, Great Ormond Street.

By this time there was considerable enlargement of the cervical lymph nodes and one in the submaxillary region appeared to be attached to bone. No other glands were palpable in the axillae or epitrochlear regions and the liver and spleen could not be felt. There was a purulent nasal discharge and the soft palate appeared lower than normal. In addition to bilateral proptosis the ocular fundi now showed the appearances of primary optic atrophy. The haemoglobin



Fig. 1. Soft tissue radiograph showing tumour arising from posterior wall of nasopharynx.

had fallen to 13.2 g% but the leucocytes remained entirely normal. X-rays of the chest and skull showed no change but a view taken to show soft tissues demonstrated a mass encroaching on the posterior wall of the pharynx (Fig. 1). It was thought possible that the child might have a retro-pharyngeal sarcoma or secondary deposits from a neuroblastoma.

An examination under anaesthesia carried out by Mr. A. W. Halfhide revealed a fixed mass in the left naso-pharyngeal area which extended anteriorly to the middle of the nasal fossa and posteriorly to the free border of the palate. A biopsy was obtained which confirmed the presence of a malignant tumour but its nature remained uncertain.

During the next two weeks the proptosis increased. His nose became blocked on the left side and oedema of the left side of the face developed. Signs of pneumonia appeared at the base of the right lung and he died the following day just two months after the original onset of blindness.

## Autopsy report

### *External appearances*

The body was that of a slight, wasted boy 108 cm in length and 15.2 kg in weight, showing generalised pallor. The chief abnormalities affected the head. There was a slight, ill-defined, subcutaneous eminence over the supra-nasal region, together with marked hypertelorism, the nasal bridge being broad and flattened and the interocular distance 4 cm. Gross bilateral exophthalmos was present with some divergence of the ocular axes and severe chemosis. The periorbital tissues were thickened, the face broad and puffy and there was some circumocular ecchymosis. In the buccal cavity, the lateral portions of both upper alveolar margins were considerably swollen and encroached on the hard palate. There was gross dissolution of the maxilla, the teeth embedded in the soft tissue were quite loose. In addition there was enlargement of the lower alveolar margins, with considerable mobility of the teeth and gingival bleeding.

### *Internal appearances*

**Primary tumour.** The nasopharyngeal lumen was reduced to a slit-like cavity by a sheath of submucous tumour tissue, most massive posteriorly where it attained a depth of 2 cm (Fig. 2). Inferiorly, the tumour extended into the palate, which was considerably thickened and somewhat nodular on its posterior aspect. Superiorly, the growth protruded into both nasal cavities, whilst laterally it had largely destroyed the medial orbital walls and facial bones fungating into the retro-ocular tissues and the maxillary antra. In general, the tumour was composed of pale, cream-coloured tissue of rather soft consistency but in the posterior pharyngeal region it was partly necrotic and deeply discoloured by haemorrhage. In the nasopharynx and nasal cavities the mucosal aspect of the tumour had a distinctly lobulated surface.

**Skull.** Whilst the basilar portion of the occipital bone appeared intact, the primary

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Fig. 2. Hemisected specimen, showing the nasopharyngeal tumour *in situ*.

tumour had invaded and largely destroyed the body of the sphenoid and the cribriform plate, extending over the inner aspect to form a large plaque which reached a maximum depth of 1 cm. The pituitary fossa was completely destroyed and the pituitary gland submerged in the mass of tissue. The bones of the calvarium were intact but over most of their inner aspect a thin sheet of neoplastic tissue was present deep to the fibrous layer of the dura, and in the medial frontal region externally a subperiosteal plaque of similar nature was present, continuous with that in the orbits. The coronal suture was unduly mobile, the suture line being replaced by a ridge of tumour which projected on either side.

### Microscopy

The tumour tissue in all sites showed the typical histological appearances of lymphosarcoma, the type cell being the lymphoblast.

From the primary nasopharyngeal tumour there was direct invasion and destruction

of the basisphenoid bone and extension as a thick layer beneath the dura. The sella turcica was largely destroyed and only the anterior lobe of the pituitary gland could be identified, it was of small size and embedded within a dense mass of tumour.

Multiple sections of the brain revealed diffuse lymphoblastic infiltration of the subarachnoid space and also of many of the perivascular spaces in the subjacent brain substance. Similar infiltration was present in the meninges of the lumbar spinal cord and also in the fascicles of the nerve roots.

### Problem of diagnosis

At the outset this child's blindness was thought to be due to a retro-bulbar neuritis, which is frequently bilateral in childhood, unlike the commoner condition in adults. It is one of the rarer sequelae of measles or may occur following a period of vague ill-health; there is a sudden loss of vision accompanied by blurring or frank swelling of both optic discs. Usually, there are no other abnormal neurological signs and complete recovery is the rule within a few weeks (5). Occasionally, a bilateral optic neuritis may be the presenting factor in neuromyelitis optica, but it is then commonly accompanied by signs of myelitis.

Doggart (2) makes the point that when visual failure in childhood is of neurological significance, there are usually other symptoms to suggest that the trouble is not entirely ocular. In this child we were unable to find any other signs during the first three weeks of his illness. In retrospect, the enlargement of the left cervical lymph nodes should perhaps have suggested the presence of generalised disease.

Loss of visual function may precede the ophthalmoscopic changes and this is commonly the case when there is compres-

sion of the optic nerve or chiasma by a tumour. In childhood such a tumour is commonly a "glioma" of the optic nerve or a craniopharyngioma. In the former, there is usually unilateral loss of vision accompanied by proptosis, and even when the chiasma is involved, it is usual for one eye to be more grossly affected than the other. Radiography will show enlargement of the optic foramen. Craniopharyngiomas produce a more gradual loss of vision, especially affecting the temporal fields; the fundi may show papilloedema or optic atrophy but the loss of vision is often greater than would be expected from the ophthalmoscopic appearances. Radiographic changes include destruction of the sella together with calcification above it and the signs of raised intracranial pressure.

Other demyelinating diseases or tumours affecting the optic radiations or visual cortex may rarely cause blindness, but it must be exceedingly uncommon for this to happen without other neurological signs.

It was not until this boy developed progressive enlargement of the cervical glands, together with downward displacement of the soft palate, that the true nature of the disease was suspected. X-ray examination of the skull then showed a soft tissue mass in the nasopharynx and a biopsy confirmed the presence of a malignant tumour.

### Tumours of the naso-pharynx

Whilst local symptoms such as nasal discharge, bloodstained sputum, discomfort in swallowing, nasal quality of voice and deafness (from Eustachian tube obstruction) often result from naso-

pharyngeal tumours, they are by no means the most frequent initial manifestations. In a series of 454 cases (7 of whom were children), Godtfredson (3) found that the commonest initial symptom of malignant tumours arising in the naso-pharynx was cervical glandular enlargement (32%). Next in descending order of frequency were nasal obstruction, with or without excessive nasal discharge, (30%) and impairment of hearing, sometimes accompanied by other symptoms of Eustachian canal obstruction (22%). In 16% of cases, various neurological syndromes were the mode of presentation, the most frequent of which were trigeminal neuralgia or paresis of the sixth cranial nerve. Unlike the commoner initial symptoms, which were usually an isolated finding, over half of those patients with neurological symptoms had concurrent cervical lymphadenopathy or nasal obstruction. Godtfredson describes only one case in which vision was affected, a man of 31 who, when seen because of nasal obstruction, was found to have nystagmus and impaired vision. In no instance was blindness a presenting symptom.

In a group of 132 patients with post-nasal tumour, Lambert (4) found that the chief presenting features were cervical metastases (46), nasal obstruction (28), neuralgia (26), and epistaxis (15). Less frequently the onset was marked by nasal discharge (6), dysphagia (3), proptosis (2) and involvement of the oculo-motor nerves (2). Again in no instance was blindness a presenting symptom, though it occurred once at a later stage of the disease.

A case showing many features similar to our own was discussed at the Royal Society of Medicine by Vulliamy (6). The



patient was a four-year-old boy who developed unilateral blindness with exophthalmos, and was subsequently found to have a lymphosarcoma arising in the nasopharynx.

### Lymphosarcoma in childhood

Though by no means rare, lymphosarcoma must be ranked among the less common neoplasms of childhood. There is a marked preponderance of affected males, as at other ages and with other lymphoid tumours, but no distinctive age distribution.

There is no absolute demarcation between lymphosarcoma on the one hand and lymphatic leukaemia on the other and this relationship is emphasised by the relatively frequent occurrence of a leukæmic phase during the course of the former.

Lymphosarcoma is one of the most invasive of tumours and its aggressiveness is emphasised by a very rapid evolution in the majority of instances. Apart from actual metastatic dissemination, there is evidence to suggest that the neoplastic process may be multicentric or systematised in its origin. In view of these features, any attempt at delineation of the sites of "primary" growths is to some extent arbitrary; nevertheless, information on this point has a certain clinical value, in differential diagnosis and elsewhere.

Children with lymphosarcoma usually come under medical attention in one of

three ways: with respiratory and/or vascular disturbances from a mediastinal tumour; with an abdominal mass which may be retroperitoneal, or alternatively involves the bowel wall and frequently leads to intussusception; or with enlargement of a group of superficial lymph nodes, usually cervical in situation. Whilst these are the main modes of presentation, occasional cases of lymphosarcoma may come under observation for a variety of reasons, such as enlargement of salivary glands or testes, subcutaneous masses, etc. (1). In the instance described the primary tumour occupied an uncommon situation and gave rise to most unusual manifestations.

### Summary

1. An unusual case is described of a boy with lymphosarcoma of the nasopharynx who presented with blindness.
2. The problems of diagnosis are discussed.
3. The features of nasopharyngeal tumours and of lymphosarcoma in childhood are briefly reviewed.

### Acknowledgements

It is a pleasure to acknowledge our gratitude to Dr. Paul Sandifer and Professor A. A. Monerieff for permission to publish this case. We are indebted to Mr. Derek Martin for the illustrations.



## References

1. DARGEON, H. W.: Lymphosarcoma in childhood. *Advance Pediat*, 6: 13, Year Book Publishers Inc., 1953.
2. DOGGART, J. H.: The neuro-ophthalmological aspects of failure of vision in childhood. *Proc Roy Soc Med*, 47: 489, 1954.
3. GODTFREDSON, E.: Sarcoma of nasopharynx. *Acta Otolaryng (Stockh), Suppl.*, 59, 1944.
4. LAMBERT, V.: Treatment of carcinoma of nasopharynx. *Proc Roy Soc Med*, 47: 547, 1954.
5. MEADOWS, S. P.: Failure of vision in childhood. *Proc Roy Soc Med*, 47: 497, 1954.
6. VULLIAMY, D. G.: A case of nasopharyngeal sarcoma. *Proc Roy Soc Med*, 41: 452, 1948.

Submitted Aug. 13, 1960

Accepted Dec. 1, 1960

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## CASE REPORT

### Salmonella Septicemia and Aplastic Crisis in a Patient with Sickle-Cell Anemia

by HERCULES MEGAS, EVANGELIA PAPADAKI and BASIL CONSTANTINIDES

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The erythroblastopenic or aplastic crisis occurring either in patients with an established hemolytic process or in hematologically normal persons is a well-documented syndrome (3, 4, 5, 6, 9, 10, 13). Vague illnesses, suggestive of respiratory infections, proven infectious agents, drugs and toxic agents have been shown to trigger its onset.

It is the purpose of this communication to report a case of aplastic crisis in a patient with sickle-cell anemia during the course of a septicemia due to *Salmonella typhimurium*.

#### Case Report

G.N., a 4-year-old boy, known to have had sickle-cell anemia since the age of 30 months, was admitted to the hospital with complaints of low grade fever, generalized malaise and anorexia of two weeks duration. The patient's 13-month-old sister remained well.

The past history revealed that the patient had had pertussis at the age of 2 years. He experienced joint pains for the first time at the age of 30 months and the diagnosis of sickle-cell anemia was established. He was never transfused nor was he observed to be jaundiced at any time.

Physical examination revealed a well-developed child who appeared moderately ill. Temperature 38°C, pulse 130, respiration 32. The sclerae were slightly icteric and the mucous membranes pale. Examination of

the chest revealed no abnormalities, except for a soft apical systolic murmur. The liver was palpable 2 cm below the right costal margin and the spleen 2½ cm below the left costal margin. No joint changes or neurological abnormalities were present.

**Laboratory data.** The urine was normal except for 1+ albuminuria. The hemoglobin concentration was 5.3 g/100 ml. The blood contained 8000 wbc/mm<sup>3</sup> with a differential count of 48% nonsegmented neutrophils, 7% segmented neutrophils, 4% eosinophils, 2% monocytes and 39% lymphocytes. A sickle preparation was positive. Fetal hemoglobin was 13%. Before transfusion paper electrophoresis of hemoglobin revealed only S type hemoglobin. Total serum bilirubin was 1.5 mg/100 ml with direct 0.0 and indirect 1.5 mg/100 ml. A direct Coombs test was negative. Chest film was reported as normal.

The patient was thought to have a painful hemolytic crisis. However, the reticulocytes were not counted, nor was special attention paid to the peripheral smear regarding the degree of polychromasia.

**Course.** On the day of admission the patient was given 160 ml of fresh blood, sedatives and aspirin. The hemoglobin level on the next day was 8.8 g/100 ml. From the second hospital day he was given 30 mg prednisone daily. For the first four days of his hospital stay he ran a low-grade fever and was complaining of abdominal and joint pains. Thereafter he started spiking a high fever, his jaundice increased and the abdominal pain became more intense. His spleen ceased to be pal-

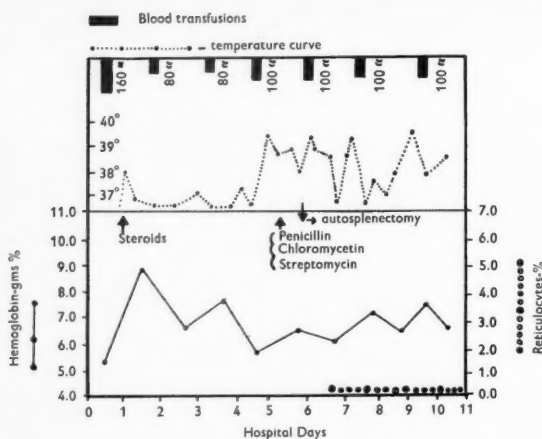


Fig. 1. Graphic representation of the clinical and hematological course.

pable, however, the liver was constantly enlarging and became extremely painful on palpation. A blood culture was obtained following which treatment with penicillin, streptomycin and chloramphenicol was begun. On the 7th hospital day the total bilirubin was 7.8 mg/100 ml (direct 5.1 and indirect 2.7 mg/100 ml). The urine showed a 3+ reaction for urobilinogen. Other studies included cephalin flocculation 3+ and thymol turbidity 1+; peripheral blood contained 3000 wbc/mm<sup>3</sup> with a differential of 12% segmented neutrophils, 23% nonsegmented neutrophils, 2% eosinophils, 6% monocytes and 57% lymphocytes. The reticulocyte count was 0.0% and the peripheral blood smear contained numerous target cells and sickle cells; one nucleated red blood cell per 100 white blood cells was counted; and thrombocytes were decreased. The marrow was cellular, but only a few orthochromatophilic normoblasts were seen. The granulocytic and megacaryocytic series showed a shift to the left, and the E:G ratio was 1:15. A moderate increase in plasma and reticulum cells was present. No giant pronormoblasts were seen.

During the next three days the general condition of the patient rapidly deteriorated. High spiking fever continued as did severe

abdominal pain mostly in the right upper quadrant. The liver was palpable 8 cm below the right costal margin and extremely painful. Digitalization had no effect upon the liver size. At no time during the clinical course did the patient appear to have heart failure, nor were abnormal focal neurological findings detected. On the 11th hospital day the patient became disoriented and expired late in the afternoon of the same day. Throughout the clinical course the hemoglobin level was maintained above 5.8 g/100 ml by fresh blood transfusions. At no time was he given more than 200 ml of blood daily. It was of interest to observe that the hemoglobin level on the day after transfusions fell again to pre-transfusion levels (Fig. 1).

*Salmonella typhimurium* was cultured from the blood which was obtained on the fifth hospital day but was not reported until two days after the patient's death.

### Discussion

In some way salmonellosis seems to be specifically related to sickle-cell anemia. Of the patients with sickle-cell anemia studied at John Hopkins Hospital, the great majority of bacteremias were due to

salmonella organisms (1). In 1951, Hodges & Holt (7) pointed out the correlation between salmonella osteomyelitis and sickle-cell anemia. Since then, there have been an increasing number of reports pointing out this correlation not only for patients with sickle-cell disease but other hemoglobinopathies as well (14). To explain this occurrence several factors have been mentioned; capillary thrombosis in the gastrointestinal tract may predispose to invasion by the intestinal organisms. Debility and "autosplenectomy"<sup>1</sup> in these patients may decrease their resistance to infection; once in the blood stream, salmonella organisms may lodge in necrotic and ischemic areas resulting from the sickling process; decreased local resistance due to ischemia may permit the growth of dormant organisms, thus explaining the observation that sickle-cell crisis often precedes symptoms of osteomyelitis (17).

Because serial reticulocyte counts and bone marrow studies have not been reported in these particular patients, the frequency of aplastic crisis remains unknown.

Chernoff & Josephson (3) reported the first case of aplastic crisis in a patient with sickle-cell anemia and salmonella cholerae suis bacteremia. A possible second case was reported by Silver *et al.* (14). In retrospect our case is of unique interest because of the many factors involved. In addition to the previously described course, it appears that in the middle of the septic course, an "autosplenectomy" took place, the liver increased in size and the patient became more jaundiced.

The effect of cortisone and similar adrenocortical steroids in the initiation and progress of bacterial infections remains a controversial issue. It has been suggested that relatively small doses may increase resistance to infection in animals (11, 12). However, the vast weight of evidence suggests that cortisone and similar adrenocortical steroids decrease resistance to infection (2). Although cortisone has been found to be of value as an adjunct in the treatment of typhoid fever (18), it has not been shown if hormone therapy should be used in other salmonella infections (15). In experimental salmonellosis it has been shown that, when hormones were administered before inoculation of the rat with sal. typhimurium, cortisone increased the mortality even though chloramphenicol had been given at the time of infection. The rapid deterioration of the general condition of our patient may be related to the use of steroids coupled with the "autosplenectomy". His death was mainly attributed to a overwhelming salmonella typhimurium septicemia.

In our case steroids did not have a favorable effect upon the evolution of the aplastic crisis. Furthermore, they did not prevent the emergence of an aplastic crisis in a patient reported by Silver *et al.* (14). Steroids were found to be of no real value in the management of aplastic crises in two patients reported by Miesh *et al.* (9). For these reasons as well as the experimental studies the use of steroids may be contraindicated in patients with sickle-cell anemia during crisis. Steroids may alter the host resistance sufficiently to provide a foothold in these particular patients for salmonella septicemia or they

<sup>1</sup> Due to fibrosis, the spleen of patients with sickle cell disease is reduced to a tiny mass, thus in this respect these patients practically have no spleen ("Autosplenectomy").

may decrease the host's resistance to an already existing salmonella infection.

Aplastic crises in patients with sickle-cell anemia have been reported to occur during the course of viral pneumonia (13), mesenteric adenitis (9), vague upper respiratory infections (3), and salmonella infection (3). Our patient is the third case in which salmonella infection served to trigger the onset of aplastic crisis and makes the salmonella organism the most frequently proven infectious agent responsible for the establishment of an aplastic crisis in patients with sickle-cell anemia.

Because of this association a blood culture should be obtained in every patient with sickle-cell anemia during crises, to rule out salmonella infection.

From the experience reported in the literature (3, 6, 13) infectious processes appear to be the most frequent cause of aplastic crises. The pathophysiologic mechanisms responsible for the establishment of such crises in patients with an established hemolytic process have been discussed by Owren (10) and Singer *et al.* (13). Hypersplenism is not thought to be responsible, either in inhibiting the marrow or in causing increased hemolysis of the red cells. The "autosplenectomy" that occurred in our patient did not favorably effect the evolution of the aplastic crisis.

The cessation of red-blood-cell production during crisis is a reversible phenomenon. The bone marrow will recover, providing that the patient survives a sufficient length of time. Lack of polychromasia and reduction or complete absence of reticulocytes in the peripheral blood of a patient with an established hemolytic process are suggestive of aplastic crisis. If

such a crisis exists, an increasingly severe anemia will occur and the bone marrow will show an impressive erythroid hypoplasia.

Basophilic cells with a diameter up to 60 in the erythroid series have been described during crisis; these closely resemble pronormoblasts in nuclear structure and staining characteristics (6, 8). Such cells were not noted in the marrow of our patient. In addition to the reticulocytopenia in the peripheral blood, leucopenia and/or thrombocytopenia may also be seen. This suggests the possibility that leucopenia and thrombocytopenia reflect an interference with the maturation of these elements.

During an aplastic crisis in patients with an already established hemolytic process, the indirect serum bilirubin remains constant or may even fall. Occasionally the rate of hemolysis may increase (16), resulting in an increased indirect bilirubin. In our cases, both the indirect and the direct bilirubin increased while the patient was in crisis. This was attributed to liver damage due to salmonella septicemia and to the sickling process, although an actual increase in hemolysis could not be excluded.

Aplastic crisis is a transitory cessation of red-blood-cell production, lasting 7 to 14 days. In patients with a hemolytic disorder such an event is of serious import. During this period blood must be given to maintain the hemoglobin values above the critical level. The underlying infectious process that triggered the onset of the aplastic crisis has to be treated accordingly. It is not known if the successful management of the infectious process will shorten the duration of the crisis.

From our own experience (to be pub-

lished elsewhere) the bone marrow recovery from the aplastic episode depends upon time and not upon the clinical course of the underlying infectious process.

### Summary

A case of aplastic crisis in a child with sickle-cell anemia and salmonella typhi-

murum septicemia is reported. Salmonellosis appears to be the most frequently proven infectious agent triggering the onset of aplastic crises in these patients. Steroids are of no real value in the management of the aplastic crisis. Their use in patients with sickle-cell anemia during aplastic episodes may be contraindicated.

### References

1. BENNET, I. L., JR. and HOOK, E. W.: Some aspects of salmonellosis. In *Annual Review of Medicine*, edited by D. A. RYTAND, Vol. 10, pp. 1-20. Palo Alto 1959.
2. CAUWENBERGE, H. VAN: L'influence de l'ACTH, de la cortisone et de substances dites ACTH produisant sur l'évolution des infections à *Salmonella* chez le rat. *Acta Clin Belg*, 2: 389-407, 1956.
3. CHERNOFF, A. I. and JOSEPHSON A. M.: Acute erythroblastopenia in sickle-cell anemia and infectious mononucleosis. *Amer J Dis Child*, 82: 310-322, 1951.
4. DAMESHEK, W. and BLOOM, M.: The events in the hemolytic crisis of hereditary spherocytosis with particular reference to the reticulocytopenia, pancytopenia and an abnormal splenic mechanism. *Blood*, 3: 1381-1410, 1948.
5. GASSER, C.: Akute Erythroblastpenie: Zehn Fälle aplastischer Erythroblastenkrisen mit Riesenproerythroblasten bei allergisch-toxischen Zustandsbildern. *Helv Paediat Acta*, 4: 107, 1949.
6. —: Aplasia of erythropoiesis. *Pediat Clin N Amer*, May 1957.
7. HODGES, F. J. and HOLT, J. F.: Editorial comment in the 1951 *Year Book of Radiology*, June, 1950 to June, 1951, edited by F. J. HODGES *et al.*, p. 89. Chicago: Year Book Publishers, Inc., 1951.
8. MANIOS, S.: Acute benign erythroblastopenia. Thesis, Salonica, 1956.
9. MIESCH, D., BAXTER, R. and LEVIN, W.: Acute erythroblastopenia, pathogenesis, manifestations and management. *Arch Int Med*, 99: 461, 1957.
10. OWREN, P. A.: Congenital hemolytic jaundice: the pathogenesis of the "hemolytic crisis". *Blood*, 3: 231-248, 1948.
11. ROBINSON, H. J., MASON, R. C. and SMITH, A. L.: Beneficial effects of cortisone on survival of rats infected with *D. pneumoniae*. *Proc Soc Exp Biol Med*, 84: 312-314, 1953.
12. ROBINSON, H. J.: Adrenal cortical hormones and infection. *Pediatrics*, 17: 770-780, 1956.
13. SINGER, K., MOTULSKY, A. G. and WILE, S. A.: Aplastic crisis in sickle-cell anemia: Study of its mechanism and its relationship to other types of hemolytic crises. *J Lab Clin Med*, 35: 721-735, 1950.
14. SILVER, H. K., SIMON, J. L. and CLEMENT, D. H.: *Salmonella* osteomyelitis and abnormal hemoglobin diseases. *Pediatrics*, 20: 439-447, 1957.
15. SPINK, WESTLEY: ACTH and adrenocorticosteroids as therapeutic adjuncts in infectious diseases, Part I. *New Engl J Med*, 257: 979, 1957.
16. The mechanism of the crises in familial hemolytic jaundice, Editorial, *Ann Int Med*, 28: 1193, 1948.
17. VANDEPITTE, J., COLAERT, J., LAMBOTTE-LEGRAND, C. and PERIN, F.: Les ostéites à *Salmonella* chez les sicklanémiques: à propos de 5 observations. *Ann Soc Belge Méd Trop*, 33: 511-522, 1953.
18. WOODWARD, T. E. *et al.*: Treatment of typhoid fever. II. Control of clinical manifestations with cortisone. *Ann Int Med*, 34: 10-19, 1951.

Submitted Nov. 19, 1960  
Accepted Dec. 12, 1960

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CASE REPORT

## Tuberous Sclerosis in a Mother and Her New-Born Son

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Tuberous sclerosis or Bourneville's disease is characterized by the symptom triad of sebaceous adenoma, epilepsy, and mental deficiency. Studies of families have shown it to be an inherited condition in which morphologically well-defined tumour-like foci occur in a number of organs; they can often be strikingly demonstrated in the kidneys, heart and retina. The disease is not confined to any particular germ layer, but may affect one or several. The progressive nature of the cerebral lesion is reflected in the increasing degree of mental deficiency and progressive epilepsy; although less characteristic skin lesions have been described, the sebaceous adenomas are regarded as pathognomonic.

The requirement of the above-named triad has prevented the diagnosis of tuberous sclerosis in many atypical cases. However, by follow-up of these atypical cases it has been possible to trace a simple dominant character in up to 4 generations in many families (5, 8, 9). No hereditary factor has been demonstrated in most of the cases published in the literature, however, and it is probable that isolated cases do occur.

About 10% of the cases in the literature concern children under 3 years of age, and almost all of these have been diagnosed at necropsy (4). This is surprising since tuberous sclerosis is a congenital condition that usually makes its appearance before the age of 2 years (3, 5). It is explained by the facts that the first sign of illness is commonly an epileptic fit; that a mental defect may be difficult to assess in early childhood; that the initial skin lesions are uncharacteristic with regard to appearance and localization, the sebaceous adenomas not becoming apparent until a later date; and that the full clinical picture develops gradually, and progresses slowly with increasing age.

The present paper is devoted to an account of 2 cases of tuberous sclerosis, in a mother and her child. They are interesting because of the direct inheritance which they illustrate, and because post-mortem examination of the infant who was only one day old, revealed lesions typical of tuberous sclerosis.

*Case 1. M.M.B.* (Record no 144/60, Dept. of Neurology). The patient was a woman of 28 years. Her parents were unmarried, and the family history is therefore under-





Fig. 1. Case 1. a) Sebaceous adenoma in the face. b) Paraungual fibroma and nail changes. c) Gingival hyperplasia.

Fig. 2. Case 2. Subependymal nodule in the wall of one of the lateral ventricles. Magnification  $10\times$ .

tain. Her father's cousin was said to have major epileptic fits. See below regarding the patient's son.

The patient had an 8-year elementary-school education, after which she had worked as housemaid until she married at 27 years of age.

Past illnesses included infectious hepatitis at the age of 10 years. Since the menarche had not occurred at 17 years, hormone treatment was instituted and continued for 2 years, at which time the periods became regular and apparently normal.

From about the age of 17 the patient suffered from fits; thus, these made their appearance at about the time hormone treatment was commenced. The patient states the fits start with dizziness and a feeling

of fainting; she has no memory of what happens during the next 30-60 seconds. Although the attacks may occur at any time, for example when the patient is drying dishes, when she is out cycling, or when attending to a child, she is able to continue her work, the only outward sign being pallor and flushing of the face. Without treatment she had up to about 10 fits per day but following treatment with phenantoin and phenobarbital which was started in 1953 they have occurred only at long intervals. There have never been convulsions during these fits; she has never fallen to the ground, has never been incontinent of urine or faeces, nor has she ever bitten her tongue.

The patient became pregnant in 1959, the date of her last menstrual period being

March 31. After an essentially normal pregnancy she was delivered on January 22, 1960, about 2 weeks after the expected date of confinement. During and after delivery the patient lost 1800 g of blood. The placenta, which was rigid, was manually removed.

On admission to the neurological department on April 19, 1960, the patient was obese and of average height. Over the bridge of the nose, the alae nasae, and the cheeks adjacent to the nose were a number of yellowish-white swellings up to the size of a rice grain (Fig. 1a), the appearance of which was typical of sebaceous adenoma. There were a number paraungual, reddish, soft swellings up to the size of a pea (Fig. 1b). At the level of each tumour there was a deep groove in the nail itself. The morphology of the swellings proved to be the same as that seen in paraungual fibromata. The papillae of the tongue were slightly enlarged. The gum behind the upper incisors was deeply and irregularly lobulated, the surface having the appearance of normal gum mucosa (Fig. 1c). The histological appearance was that of non-specific hyperplasia and inflammation, and was probably not due to tuberous sclerosis (the patient had received phenantoin). The hard palate was unusually high and narrow, but otherwise there were no abnormal findings in the ears, nose, or throat. The distribution of hair was normal. The abdomen was rather adipose, with striae gravidarum. No abnormalities of the internal organs were noted. The results of oto-neurological and ophthalmoneurological examination were normal.

Experimental psychological examination showed that the patient's capacity in most of the tests was under the average. The patient's intelligence was obviously subnormal.

The EEG was pathological, with moderately marked, continuous, non-specific abnormality in the right fronto-temporal region.

X-ray of the skull, including tomography, disclosed slight to moderate hyperostosis

frontalis interna. Within the right frontal region there were extensive plaque-like patches of calcification in an area slightly larger than a walnut. There were also suspicions of similar scattered plaques in both parieto-temporal regions.

X-rays of the chest and abdomen were normal. The ECG was normal. The routine blood and urine tests gave normal results except for slight anaemia (Hb 10.7 g%). The Wassermann, Kline, and Meinicke reactions were negative. The Sabin-Feldman dye test and the complementfixation test for toxoplasmosis were negative. The patient's bloodgroup was O, D-negative.

*Case 2.* (Record no 120/60, Dept. of Paediatrics). This patient, a boy, was the first and only child of Case 1. The expected date of birth was January 7, 1960; he was born on January 22, 1960, in right occipito-anterior presentation, and weighed 3,840 g. The amniotic fluid was deeply stained with meconium. The mother had fits during labour, and was therefore given pethidine (50 mg) and sodium amobarbital (0.2 mg). Slowing of the foetal heart was noted several times during the hours before birth.

Immediately after birth the infant was in good condition after aspiration of mucus. An attack of deep cyanosis took place 1½ hours after birth, and he was transferred to the paediatric clinic. On admission to the ward he was cyanotic and hypotonic. Respirations were shallow and infrequent, with dyspnoea and a piercing cry. Temperature 32.5°C. There were no skin lesions. Large quantities of mucus were aspirated. The breath sounds were abnormal with crepitations on both sides. Repeated attacks of cyanosis took place during the next few hours, and the infant died during such an attack 11 hours after birth.

Laboratory tests. Estimation of barbiturate as amobarbital in certain organs post mortem: serum 0.8 mg/100 ml; brain 0.6943 mg/g wet weight; liver 0.023 mg/g wet weight.

*Necropsy.* (Record no 56/60, Dept. of Pathology).

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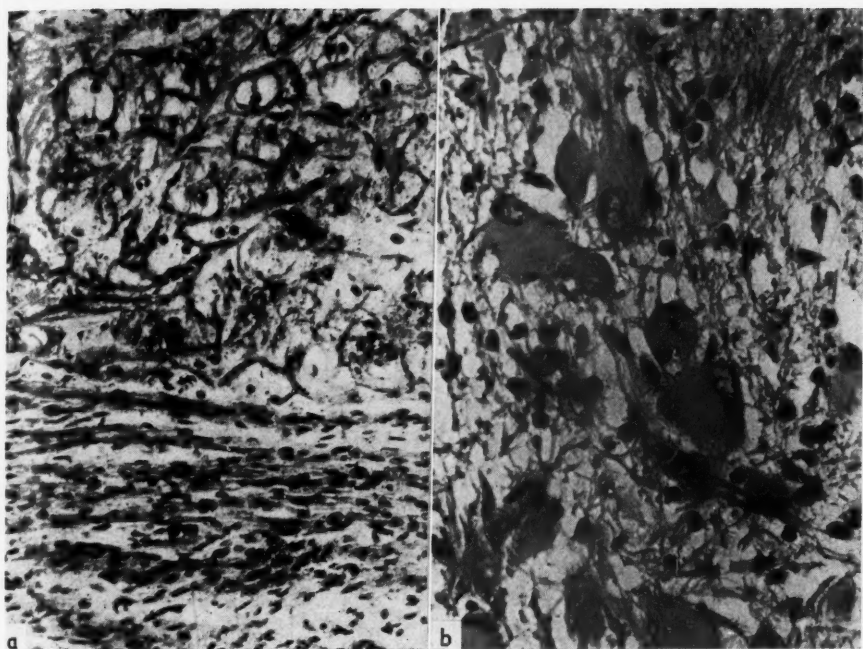


Fig. 3. Microphoto of the myocardial and cerebral changes in Case 2. a) Myocardium with changed muscle fibers in the upper part of the photo. Magnification  $170\times$ . Haematoxylin eosin stain. b) Brain nodule with gemistocyte-like cells. Magnification  $450\times$ . Holzer stain.

#### Cardiovascular system.

Heart weight 24 g. There were numerous subpericardial petechiae. On the outside of the anterior wall of the left ventricle there were two slight nodules the size of a pea arising from and only slightly paler than the muscle. Three similar pin-head size nodules were present beneath the endocardium on the septal wall of the left ventricle.

Microscopical examination of the nodules disclosed diffusely delimited changes in the muscle fibres, including vacuolization and fusiform enlargement (Fig. 3a), transverse striations demonstrable at isolated areas only, moderate quantities of glycogen, and no fatty deposits.

**Respiratory tract.** A few petechiae were present on both pleurae. The lungs had the consistency of liver and were dark red in color. Microscopical areas of atelectasis

were demonstrated and, in certain areas, small foci of bronchopneumonia.

**Nervous system.** The brain was of normal size, and no lesions were visible on the surface of the cortex. Small subependymal swellings up to about 5 mm in diameter were present in both lateral ventricles along the course of the terminal vein (Fig. 2). No other similar changes could be demonstrated at any other site in the brain.

Microscopical examination of the lesions revealed the presence of numerous large cells rich in cytoplasm, some of them multinuclear. These were probably of glial type, and some resembled gemistocytes (Fig. 3b). The lesions also contained glial cells of usual appearance; and the medulla also contained isolated, pale large cells, although no such cells were seen in the grey matter of the cortex.

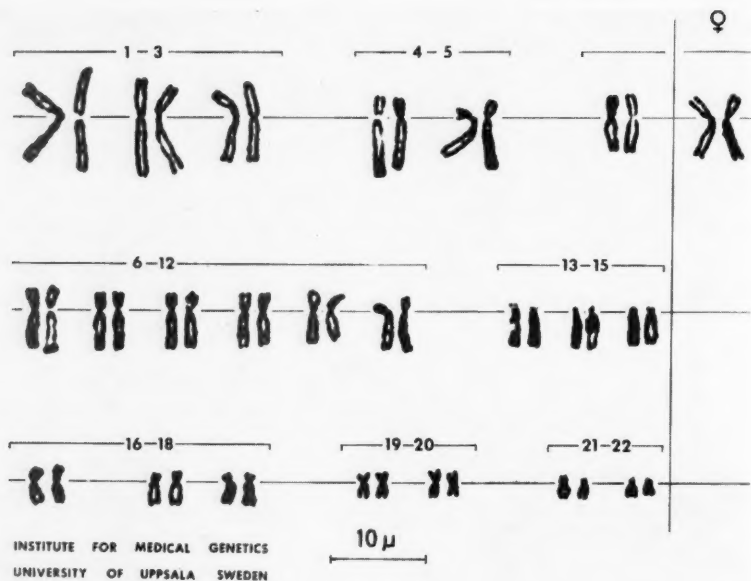


Fig. 4. Karyogram from normal skin of Case 1 showing a normal female chromosome complement.

*Spleen, lymph nodes, and bone-marrow.* Microscopical examination revealed numerous large mono- or polynuclear megakaryocyte-like cells with abundant cytoplasm. Otherwise there were no changes in these organs.

*Abdominal viscera and endocrine organs.* Microscopical examination of the liver showed either isolated, or small groups of large, pale cells with abundant cytoplasm. No other significant changes were seen in these organs.

The *placenta* showed a large infarct about 10 cm in diameter, and several small infarcts. Histological examination revealed slight vasculitis in the umbilical cord.

*Bacteriological investigation* of the post-mortem material disclosed no definitely pathogenic bacteria.

The morbid-anatomical changes, localized chiefly to the brain and myocardium, tally exactly with the changes typical of tuberous sclerosis; the cause of death was due to pulmonary atelectasis and bronchopneu-

monia. Whether or not these pulmonary lesions may be placed in relation to the primary disease, tuberous sclerosis, is impossible to say. Pneumonia would seem to be a far less common cause of death in tuberous sclerosis than the cerebral lesions (including hydrocephalus) or cardiac abnormalities (6).

#### Chromosome studies in the mother<sup>1</sup>

Biopsies from the apparently normal skin of the left thigh and from a fibroma on the nail-bed of a toe from the mother were cultivated according to the standard cell culture technique used at the Institute for Medical Genetics in Uppsala (2). Two and five primary cultures respectively, were obtained. Considering the age of the patient (28) the cultures grew normally and good cytological preparations were obtained after 24 days.

<sup>1</sup> This investigation has been aided by a grant from the Foundation's Fund for Research in Psychiatry to Professor Jan A. Böök.

The cells derived from the fibroma showed a slightly better growth, probably due to a larger initial explant.

Apparently undamaged cells from both cultures were examined cytologically. The majority of the cells had the normal diploid chromosome number (46) (cf. Table 1).

TABLE 1. *Chromosome counts.*

Tissue	Chromosome number				Total no. of cells
	44	45	46 ± 1	46	
Normal	—	—	3	10	13
Fibroma	1	—	1	30	32

Three very distinct cells derived from the normal skin and five cells derived from the fibroma were matched and analysed in detail. All these cells showed a completely normal chromosome complement (Fig. 4). None of the cultures had an abnormally high frequency of polyploid cells (0-4 per cent).

A 17 year old male with tuberous sclerosis has been karyotyped earlier at the Institute for Medical Genetics in Uppsala. Cells derived from bone marrow showed a normal karyotype with 46 chromosomes and XY complement.

### Discussion

The full triad of symptoms was present in the mother and in such cases the diagnosis does not usually present any difficulties. Classical tuberous sclerosis may be overlooked for years, however. It is therefore understandable that the first solitary symptoms in a child do not as a rule suggest this disease. In the infant the diagnosis was made solely on the basis of the post-mortem findings. To what extent the specific changes of tuberous

sclerosis contributed to the child's death is difficult to assess. The probable cause of death was atelectasis and pneumonia, presumably secondary to a long and difficult delivery, and with some effect of barbiturate in addition. Since it is known that infants dying with this disease during the first years of life usually do so as a result of heart failure, and, further, since this patient was found to have anomalies of the heart, cardiac failure may well have contributed to the development of the respiratory illness.

The fits in tuberous sclerosis, which commonly first occur before the age of 2 years, do not differ in any way from fits of other cause, and therefore do not give any clue to the diagnosis except as part of a typical symptom complex. If tuberous sclerosis is to be excluded in a child with fits the physician must base his opinion on other findings. The skin lesions do not acquire the characteristic appearance of sebaceous adenoma until the child has reached school age, although other less typical skin anomalies are commonly seen during the first years of life (3). The presence of renal malformations does not as a rule provide a clue, as these are often clinically non-specific and silent until so late that the neurological and cutaneous manifestations have already indicated the diagnosis.

Some help can be obtained from roentgenological and electrocardiographic investigations, however. Calcification can occasionally be demonstrated in the cerebral foci, and in not a few cases the presence of foci has been shown by air encephalography (7). Furthermore, during recent years typical roentgenological changes in the lungs and bones have been

described in advanced cases (1). A corresponding change, phakoma, is seen in the retina. Death from cardiac failure is common (4) in young patients. The myocardial lesions may cause cyanosis, enlargement of the heart, tachycardia, and electrocardiographic anomalies.

### Summary

Changes typical of tuberous sclerosis were observed in a 28-year-old woman

and her one-day-old son. The clinical signs in the woman were skin lesions, epileptic fits, and low intelligence. In the boy the diagnosis was based on post-mortem findings, notably changes in the heart and brain. The diagnostic criteria of this disease are discussed. No chromosomal abnormalities were found either in the normal skin nor in the fibroma on the nail-bed of the mother.

### References

1. ASHBY, B. W., RAMAGE, D.: Lesions of the vertebrae and innominate bones in tuberous sclerosis. *Brit J Radiol*, 30: 274, 1957.
2. BÖÖK, J. A., FRACCARO, M., and LINDSTEN, J.: Cytogenetical observations in mongolism. *Acta Paediat (Upps)*, 48: 453, 1959.
3. CRAO, D. H. C.: Congenital neurocutaneous syndromes in childhood. *J Pediat*, 55: 447, 1959.
4. DEBRÉ, R., THIEFFRY, S., MOZZICONACCI, P., BARGETON, E. and RAMADE, J.: La sclérose tubéreuse de Bourneville chez le nourrisson et le petit enfant. *Arch Franc Pédiat*, 9: 342, 1952.
5. ENGLISH, D. A.: Tuberous sclerosis. *Arch Paediat*, 66: 24, 1949.
6. HALLERVORDEN, J. and KRÜCKE, W.: Die tuberöse Hirnsklerose. *Handbuch der Spez. Path. Anat. und Histol.* 13/4: 602, 1956.
7. ILLING, E.: Pathologisch-anatomisch kontrollierte Encephalographien bei tuberöser Sklerose. *Z Ges Neurol Psychiat*, 176: 160, 1943.
8. LAHDENSU, S.: A case of tuberous sclerosis. *Acta Paediat (Upps)*, 34: 1, 1947.
9. MARSHALL, D., SAUL, G. and SACHS, E.: Tuberous sclerosis. *New Engl J Med*, 261: 1102, 1959.

Submitted Dec. 20, 1960

Accepted Dec. 30, 1960

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PROGRESS IN PEDIATRICS

## The Diagnosis and Course of Rheumatoid Arthritis and Benign Aseptic Arthritis in Children<sup>1</sup>

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Arthritis and arthralgias are not rare symptoms among patients in a pediatric ward. In some the etiology is obvious as in cases of *septic* infectious arthritis. In others, however, the etiology and pathogenesis may be more obscure, the joint affection frequently following an acute upper respiratory infection. In these *aseptic* conditions an allergic-toxic mechanism seems probable and the affection may be labelled postinfectious arthritis. Rheumatic fever can be regarded as an entity within this group, the heart involvement being the outstanding feature and the decisive factor for the prognosis. It appears that an allergic-toxic mechanism can also be implicated in arthralgias which occur in some cases of anaphylactoid purpura and in the arthritis, which is one of the extraintestinal manifestations of ulcerative colitis.

Rheumatoid arthritis (RA) can be regarded as an aseptic arthritis of unknown origin which runs a prolonged course. The clinical features in children which vary in their pattern from an insidious and

undramatic picture to a fullblown, sepsis-like condition, represented by the syndrome of Still and Wissler's "subsepsis allergica" (28, 30, 33, 34), have been confirmed in several recent reports (8, 9, 17, 22, 25, 29). It is generally agreed that most cases in children show certain common trends when compared to adults with the disease. These include the greater tendency for larger joints to be affected early in the course, the common acute onset, the relatively high incidence of visceral symptoms and eye lesions, and the difficulties in demonstrating the rheumatoid factor in the serum (35, 36).

The diagnosis in a child presenting a long-standing, symmetrical, aseptic polyarthritis with or without systemic signs hardly offers any difficulties, the only problem being to differentiate the condition from other more malignant collagen diseases such as disseminated lupus erythematosus. On the other hand, there may be difficulties at the other end of the spectrum of severity in demarcating RA from other more benign conditions, variously labelled as "postinfectious" arthritis or nonspecific synovitis. These children do not have severe extraarticular systemic

<sup>1</sup> The study was made possible by a grant from Förestamajblommans Riksförbund mot Foltjukdomarna, Gothenburg, Sweden.



manifestations, relapses may occur, but the joint process is not destructive and no permanent disability results. It can be claimed that these represent unusually mild cases of RA; however it seems more reasonable to demand certain minimal diagnostic criteria in order to be able to compare experiences from different clinics. The well-known diagnostic criteria for RA in adults elaborated by the American Rheumatism Association (6, 25) are not applicable without modification when dealing with children. In this investigation an attempt has been made to establish criteria, special attention being paid to the characteristic features of the disease in childhood, and to try to present them in a simplified and perhaps more surveyable way. A clinical and prognostic comparison has been made between cases fulfilling these criteria and cases not fulfilling them; the latter have in this report been labelled benign aseptic arthritis (BAA).

Prognostic studies of RA in children have yielded varying results, to a large extent depending on a differing selection of the material. Since 1948 the experience from several large clinics has been reported. Bille's series comprised 65 children (4) with a disease of long duration. In spite of this the majority improved following chrysotherapy indicating some reversibility of the condition. Schlesinger's material (26) consisted of 20 cases with acute onset and high fever, showing the features of Still's disease. Complete recovery with no obvious residual arthritis was the final result in three, varying degrees of residual defects remained in 11, four had died and in two the disease was still active. Sury in 1952 published a detailed report on 151 cases (29). The mean observation time was 12 years and at the follow-up examination 39 per cent had healed without defects, 32 per cent had residual lesions, not severely

handicapping the patient, whereas 29 per cent were seriously invalided and 8 per cent had died. The prognosis seemed to be worse in cases with an acute onset. Barkin also in 1952 reported 51 cases (3) with onset before 12 years of age with a remarkable incidence of significant heart disease (31 per cent); Still's severe type of juvenile RA was present in about 35 per cent. A relatively large proportion of Barkin's cases were serious as reflected in his pessimistic dictum "once an arthritic always an arthritic". Following an observation time varying from a few up to 40 years after hospitalization, 20 per cent had died, 39 per cent were incapacitated and 41 per cent had a functional recovery permitting performance of all or almost all normal activity. In 1955 Fyfe reported on 72 children with RA (12), 56 of whom had a recent follow-up examination. The duration of observation varied from a few to 25 years. In 61 per cent of the examined children the disease had not shown any signs of activity during the preceding two years and approximately two-thirds of these had recovered without sequelae. Less than 10 per cent of those followed-up showed a more significant handicap. An even more optimistic view was expressed by Grokoest and co-workers in 1957 (14). Of their 110 patients examined an average of nine years after onset of the disease, eight had died, 76 per cent of the remainder had recovered, 22 per cent had a slight to moderate handicap and only two per cent were severely incapacitated. Edström & Gedda reported a prognostic study (9) dealing with 90 children. Five of them had the picture of Still's syndrome, two of whom died, one became invalided and two recovered. The 85 other cases, called juvenile rheumatoid arthritis, showed complete recovery without residual symptoms in 62 per cent, whereas 31 per cent showed varying degrees of incapacity. One child died and five showed persistent signs of activity rendering a definite evaluation of the outcome difficult. Norcross *et al.* collected 62 cases published in 1958 (21) with an observation time in all of them exceeding seven years. The average duration of activity was 66

months. Two-thirds had only one period of activity, but recurrences had been observed following an interval of 20 years. The disease was inactive in 73 per cent at the follow-up examination. Two thirds had recovered entirely or almost completely, the rest showing varying degrees of handicap. Close to five per cent had died. The 16 children with the clinical picture of Still's syndrome had the worst prognosis. Sairanen, in 1958, published a report dealing mainly with the roentgenological aspects of 100 patients with juvenile RA (25). His material apparently represents a selection of more chronic and disabled cases. Twenty-two were considered arrested and 15 of these were largely incapacitated after an average duration of 13 years. Köttgen & Callensee in their monograph (19), based on inquiries from different hospitals, found that at discharge the disease was still active in three fourths of the patients. No follow-up examination was performed. Ansell & Bywaters, in 1959, reported on their vast experience of more than 200 cases of juvenile RA (2). Thirty-one per cent showed a more or less continuous course and 22 per cent an intermittent one. Close to half of them had no residual activity of the disease; the active period in these cases had been an average of 7.4 years. One hundred and sixteen of their children had a follow-up examination five years after onset of the disease. The prognosis turned out to be quite favorable with normal functional activity or only a slight handicap in 98 per cent if the patients had been seen by a physician; within one year after this onset, 44 per cent were entirely symptom free. If not seen during the first year the corresponding figures were 61 and 13 per cent. From the follow-up 10 years after the onset it was concluded that some further improvement could in general also be seen during the second five-year period.

In the investigations mentioned above the varying results can easily be explained by the differing geographical and social factors, diverging principles of selection

and heterogenous classification. The distinction from other types of aseptic arthritis has sometimes not been well defined.

### Present investigation

Records of children with arthritis treated in pediatric departments of four Stockholm hospitals from 1952 to 1957 were collected for a preliminary screening. The following were eliminated from the study: patients, whose total duration of joint symptoms was shorter than three weeks; those where a septic etiology could be proved or seemed probable or where rubella had preceded the symptoms; children with allergic purpura, ulcerative colitis, psoriasis, leukemia, other collagen diseases, or when the criteria for the diagnosis of rheumatic fever according to Wallgren 1957 (31) were fulfilled. Ninety-one remained after these cases had been excluded. During the period July 1959 to March 1960 eighty-four of these children were examined by the author; an additional three were examined by other physicians. Four children could not be traced or were not willing to cooperate. The group investigated consisted in part of typical cases of rheumatoid arthritis and partly of aseptic arthritis of a more benign character. The data from the records were collected to formulate a classification which utilized criteria described later in this paper as well as those suggested by the American Rheumatism Association for the diagnosis of rheumatoid arthritis. It must be pointed out, however, that the charts were incomplete in several instances for the obvious reason that they had not been written with these special criteria in mind. The subsequent course of each patient was analyzed from anamnestic data collected at the time of follow-up, from a complete physical examination and, in some cases, from X-ray and laboratory studies.

The main diagnostic grouping of the patients was based on the following criteria.

### Major criteria

1. Objective arthritis symptoms from one joint observed by a physician and of a continuous duration of at least six weeks.

2. One or several additional joints affected according to the same qualification and with the same duration. An interval exceeding three months between the time the first and subsequent joints were affected was accepted.

3. Significant agglutination titer indicating presence of "the rheumatoid factor".

4. Typical X-ray changes.

5. Biopsy showing characteristic changes.

### Minor criteria

1. Continuing active arthritis of one joint observed by a physician for at least three months totally, or a recurrence with a duration of at least six weeks.

2. Morning stiffness.

3. Subcutaneous nodules.

4. Erythema multiforme rheumatoides.

5. Iridocyclitis, uveitis or band-shaped keratitis.

### Exclusions

Findings indicating a septic, tuberculous or viral etiology, salmonella infection, urinary tract infection, disseminated lupus erythematosus, periarteritis nodosa, leukemia, ul-

cerative colitis, anaphylactoid purpura, psoriasis, agammaglobulinemia, rheumatic fever.

Two major criteria or one major and two minor were required for the diagnosis of rheumatoid arthritis presupposing that exclusion factors were absent. Cases in this investigation not fulfilling these criteria were called benign aseptic arthritis.

### Results

Of the 87 patients, seen in a follow-up examination, 48 fulfilled the diagnostic criteria of rheumatoid arthritis (RA); the number labelled benign aseptic arthritis (BAA) was 39. These two groups were compared as to the clinical picture and course.

*Heredity.*—The incidence of different types of "rheumatic" diseases among relatives is shown in Table 1. The incidence was similar in the two groups.

*Sex ratio.*—The preponderance for girls is shown in Table 2 and was of similar magnitude in both groups.

*Age.*—The age at onset is illustrated in Fig. 1. It is clear from this that in the RA group the disease had an earlier onset,

TABLE 1. *Heredity for rheumatic diseases.*

	Mother	Father	Siblings	Other relatives (grand-parents, uncles, aunts, cousins)
BAA (39 cases):				
Acute polyarthritis, rheumatic fever				
benign aseptic arthritis	2	2	0	0
Rheumatoid arthritis	0	0	0	3
Non-classifiable	1	0	0	0
Positive heredity for all types in 21 %				
RA (48 cases):				
Acute polyarthritis, rheumatic fever				
benign aseptic arthritis	3	0	0	2
Rheumatoid arthritis	1	0	0	4
Non-classifiable	0	0	0	2
Positive heredity for all types in 25 %.				

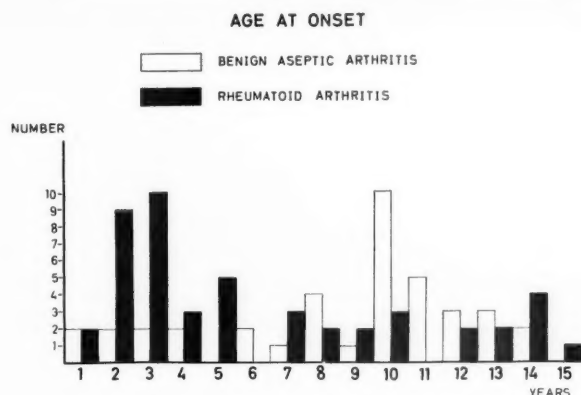


Fig. 1. Age at onset in BAA and RA.

TABLE 2. Sex distribution.

	♀	♂	♀/♂
BAA	26	13	2/1
RA	31	17	1.8/1

being most common before 5 with a peak between 2 and 3 years of age. In contrast BAA occurred more commonly in older children, the peak appearing between 10 and 11 years of age.

*Preceding infection.*—A history of an upper respiratory infection preceding the joint symptoms was a common finding in both groups. Such a history within four weeks before the onset occurred in 15 of the 39 BAA group and in 14 of the 48 children with RA. In order to analyze the importance of a preceding infection in more detail a further comparison was made regarding evidence of this association. Among children admitted within 3 weeks of the onset or recurrence of joint symptoms only a few in both groups had significant findings of an acute upper respira-

tory infection. Nose and throat cultures were performed in most of the children and the findings have been summarized in Table 3 and 4. There did not seem to be any difference as to the incidence of pathogenic organisms in the two groups, nor was the incidence influenced by the variable length of the time having elapsed between the onset of the symptom and the admission to the hospital. It is also seen that the incidence of various pathogenic organisms was almost identical in the two groups. The antistreptolysin and antistaphylococcal titers have also been

TABLE 3. Nose and throat cultures.

Hospitalization	Disease	Pathological finding	Normal finding
Within 3 weeks after onset	BAA	11	11
	RA	9	12
Later than 3 weeks after onset	BAA	7	8
	RA	11	11
Total	BAA	18	19
	RA	20	23

TABLE 4. *Pathogenic microorganisms in nose and throat cultures.*

	Hemolytic streptococci	Pneumococci	Staph. aureus	Haemophilus influenzae	Negative culture
BAA	7	5	10	0	20
RA	6	5	10	1	25

TABLE 5. *Antistreptolysin titer.*

Hospitalization	Disease	Less than 200	200-1000	Higher than 1000
Within 3 weeks after onset:	BAA	11	12	1
	RA	12	7	2
Less than 3 weeks after onset:	BAA	8	6	1
	RA	13	5	5
Total	BAA	19	18	2
	RA	25	12	7

TABLE 6. *Antistaphylolysin titer.*

Hospitalization	Disease	Less than 2	2-5	Higher than 5
Within 3 weeks after onset:	BAA	17	4	3
	RA	11	6	4
Later than 3 weeks after onset:	BAA	11	3	1
	RA	17	5	1
Total	BAA	28	7	4
	RA	28	11	5

TABLE 7. *X-ray-examination of sinuses and lungs.*

Hospitalization	Disease	Sinuses			Lungs		
		Patho- logical finding	Normal finding	Not exam- ined	Patho- logical finding	Normal finding	Not exam- ined
Within 3 weeks after onset	BAA	1	1	19	5	6	11
	RA	2	4	14	3	6	11
Later than 3 weeks after onset	BAA	1	5	12	1	9	8
	RA	2	7	19	1	9	18
Total	BAA	2	6	31	5	15	19
	RA	4	11	33	4	15	29

compared as illustrated in Table 5 and 6. The highest value within one month following the admission has been noted and the groups have again been divided into subgroups, depending on the duration of the symptoms before the admission (irrespective of it was the first onset or a recurrence). X-ray examination of the sinuses and lungs within one week after the admission was undertaken in relatively few children. The results are shown in Table 7, where the same subgrouping has been made. An X-ray-picture indicating sinusitis was found in approximately one fourth and evidence of a bronchopneumonia in one fifth. No difference between children with BAA or RA was present.

In no case had a history indicating a urinary tract infection been given. Urinary sediment from specimens obtained soon after admission showed some increase of white cells in five of the patients with RA and in two of those with BAA. When repeated examinations were performed the increase was not constant and urine cultures were sterile.

**Trauma.**—A history of trauma in the region of the first joint involved within a month preceding the onset was present in one patient with BAA and in four with RA. The figures are too small to indicate a significant difference.

**Monarticular onset.**—A monarticular onset was relatively common. In the beginning symptoms were localized to a single joint during at least one month in 19 of the 39 BAA cases and in 14 of the 48 RA cases. As time progressed this difference became very marked; only two of the children with RA remained monarticular during the entire period of observation as compared to 17 with BAA.

#### EARLY DISTRIBUTION OF JOINTS AFFECTED



Fig. 2. Joints affected in BAA and RA within two months after onset.

#### Early distribution of affected joints.

The distribution of affected joints within two months after the onset is shown in Fig. 1. The knee was most commonly affected in both groups. There was a general tendency for more joints to be affected among the RA patients and the symptoms were more often localized to the larger joints in this early stage of the disease. The cervical spine was involved in RA exclusively.

**Extra-articular manifestations.**—Visceral manifestations were rare within two months after the onset. Cardiac involvement was present in four of the children with RA, as judged by enlargement of the heart on X-ray (one case) and definitely abnormal ECG (three cases). The ECG changes consisted of a lowering of the S-T

TABLE 8. *Duration of fever.*

	BAA	RA
Afebrile	22	25
38°C or above maximum seven days	17	21
38°C or above for 8-21 days	0	0
38°C or above for more than 21 days	0	2

interval and abnormally flat T waves. The children with BAA were all normal in this respect. None of the RA patients followed a typical course for Still's syndrome and no significant enlargement of the spleen or the lymph nodes was found. There was no evidence of impaired liver function; neither cerebral symptoms nor skin rashes were noted. Involvement of the eyes occurred only among the RA children as iritis (three cases), chorioiditis (two cases) and episcleritis (one case). As mentioned earlier iridocyclitis or uveitis were included among the minor criteria for the diagnosis of RA.

*Temperature and laboratory findings.*—A comparison between the two groups as to the degree of fever, elevation of the E.S.R. and level of the serum gamma-globulin fraction within two months following the first hospital admission has been made and the findings have been tabulated (Tables 8-10). No significant difference between the two groups was found,

TABLE 9. *Sedimentation rate.*

	BAA	RA
0-14 mm	13	6
15-29 mm on at least two occasions	9	17
30 mm or above on at least two occasions	17	25

TABLE 10. *Gamma globulin in serum.*

	BAA	RA
Less than 15 %	4	1
15-25 %	3	11
Higher than 25 %	0	1
Not examined	32	35

but it must be noted that serum electrophoresis was performed in only a minority. Had this been done more systematically the tendency to higher gamma-globulin values in the RA group might have been demonstrated more definitely. A hemoglobin level lower than 10 g per 100 ml during the same early period of the disease was present in eight of the RA and in only one of the BAA patients. During the first two months after the admission the sheep cell agglutination test was performed in 24 of the RA cases, two of them being positive but with low titers (1/64). During the subsequent course additional positive titers appeared in three children (1/64, 1/128, 1/512).

*Course.*—The widely different course and the entirely dissimilar prognosis in the two groups has been visualized in various ways. The duration of the disease up to the time of the follow-up examination is presented in Table 11. The figures do not as a rule represent the duration of a continuous activity, they merely give the span of time during which the symptoms

TABLE 11. *Continuous duration or period (in years) during which relapses occurred.*

	0	1	2	3	4	5	6	7
BAA	21	10	6	1	1	0	0	0
RA	8	8	5	4	4	4	4	11



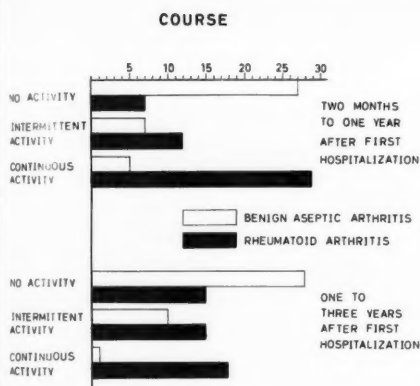


Fig. 3. The course within three years after the first hospitalization.

were present either continuously or intermittently. The typical pattern for BAA was a single bout of activity of some weeks or a few months duration although recurrences were not rare within the first two years. Relapses after this period of time occurred only twice. In contrast, in more than half of the RA patients, continuous or recurrent activity was present after three or more years. Persistent activity was present or relapses had occurred in 11 cases for as long as 7 years after the onset and it must be remembered that only a part of the material had been observed for this length of time. Thus it is probable that the number showing this long-lasting course would increase during prolonged observation.

The course during the first three years after the first hospital admission has been illustrated in more detail (Fig. 3). The more benign progress and the lesser tendency towards continuous activity in the BAA cases is obvious. In Fig. 4 the length of time the patients had been free from activity of the disease preceding the fol-

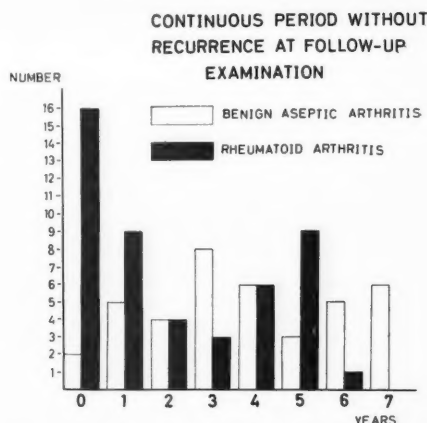


Fig. 4. Period preceding the follow-up examination without any activity of the joint symptoms.

low-up is given. The varying duration of observation again limits the possibilities for definite conclusions, but the systematic difference between the two groups of cases is clear.

In the patients who had no signs of activity for at least one year preceding the follow-up examination the incidence of residual findings was compared (Fig. 5). The sequelae include defective range of movement in the affected joints, atrophy of the muscles, deformities, contractures and growth disturbance. Half of the RA cases showed residual defects, whereas

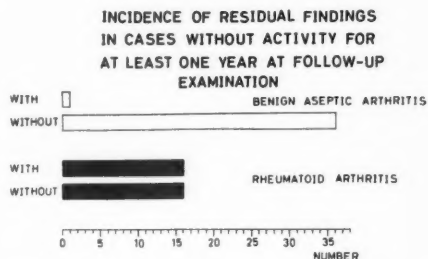


Fig. 5. Incidence of residual findings in BAA and RA at the follow-up examination.

TABLE 12. *Degree of handicap.*

	BAA	RA
<i>One year after first hospital admission</i>		
Normal activity	39	38
Partially handicapped	0	8 (17 %)
Severely handicapped	0	2 (4 %)
<i>Three years after first hospital admission</i>		
Normal activity	37	37
Partially handicapped	0	4 (9 %)
Severely handicapped	0	2 (5 %)

only one of the patients with BAA had residua. The incidence and degree handicap is given in Table 12. Here the functional state of the children has been estimated one and three years, respectively, after the first hospital admission. By normal activity is meant that the children were able to take full part in school work and outdoor activities, whereas the partially handicapped had a limited physical capacity, which, however, had not kept them from an essentially normal life. The severely handicapped had either been bedridden, confined to a wheelchair existence or at least had largely been dependent on help for their daily needs. One year after the first hospital admission 21 per cent of the RA patients were handicapped to a varying degree, as compared to none of those with BAA. Three years after the first hospital admission the functional state could be evaluated in 37 children with BAA and 43 with RA. The former were again all labelled as having normal activity, whereas 14 per cent of the latter were handicapped; one-third of this minority severely handicapped. In one of these the handicap was due entirely to seriously impaired vision, following a bilateral uveitis. It is thus evident that

the prognosis is entirely different in BAA as compared to RA also concerning these more severe and largely irreversible conditions. It must, however, be pointed out, that the time of observation is too short to establish the definite outcome, with respect to the ultimate incidence of a handicap.

### Discussion

The criteria presented above for the diagnosis of RA in children need to be commented upon. As previously mentioned the diagnostic criteria elaborated by the American Rheumatism Association do not take into account the special character of the disease in childhood. For reasons of simplicity the present classification has been designed to include a combination of criteria required for making the diagnosis highly probable; not, as in A.R.A. classification, to establish the diagnosis with different degrees of probability.

The *first* criterion in the present classification deals with signs of arthritis observed by a physician; including all objective findings or symptoms with the requirement of a continuous duration of six weeks. A monarticular RA may occur and is possibly more common in children than in adults. A polyarticular affection is, however, more typical especially if it is symmetrical. This is expressed in the *second* criterion. The A.R.A. has the same requirement of duration for the diagnosis of classical or definite RA. In the A.R.A. classification, however, it is stated that the interval between the joints becoming affected should not exceed three months. In view of the intermittent course so common in children it has been suggested

that a longer interval should be accepted. The *third* criterion is a significant agglutination titer, indicating the presence of "the rheumatoid factor". It is generally agreed that this is not a common finding in RA in children except possibly when recent methodological modifications are used. This indicates that the low incidence of positive results observed is attributable to the lack of sensitivity of the procedures rather than to an absence of the rheumatoid factor (36). On the other hand, there does not seem to be a higher incidence of nonspecific positive reactions in children, and a positive reaction, although in itself not frequent, must be accepted as valuable support for the diagnosis. Typical X-ray changes constitute the *fourth* criterion and is also found in the A.R.A. classification. What can be regarded as typical may be a matter of dispute, but this point has recently been dealt with in detail in a clinico-roentgenological study of RA in children (25). The *fifth* criterion includes all biopsies, which show characteristic changes, including microscopic findings in joint capsules and subcutaneous nodules as described in the notes to the A.R.A. classification (24). These five criteria have been evaluated as being the most important and have, consequently, been considered as *major criteria*.

The following symptoms and findings have been labelled *minor criteria*. If a monarthritis persists for as long as three months or if a recurrence has a continuous duration of at least six weeks, this is listed as the *first* because it increases the likelihood of a prolonged course and a "rheumatoid character" of the disease. One reason for regarding these prolonged and intermittent cases of monarthritis as

truly rheumatoid has been given by Sury (29), who pointed out the similar incidence of eye complications in these patients, as compared to those with a more typical polyarticular RA in children. Morning stiffness is a typical finding in RA in adults and is also fairly common in children. The present material can not be used to estimate its incidence, however, as its importance has not been realized and notes on its occurrence were made only occasionally in the records. It is the author's impression that morning stiffness is not a rare finding in other types of arthritis in children, but there is reason for indicating it as a *second* minor criterion. The *third* is the presence of subcutaneous nodules. They are not frequently encountered and may also occur in acute rheumatic fever. The *fourth* is the exanthem variously described by different authors (13, 15) as being a common phenomenon in childhood RA, especially among those running a more acute and septic course with high remittent fever. Cases of this type were not seen in this material and no convincing notes describing this rash have been found. Gauchat & May (13), however, observed it in 75 to 80 per cent of children with high fever in the initial stages. They described it as maculopapular in character and of a varying distribution similar in appearance to erythema multiforme and called it erythema multiforme rheumatoides. The *fifth* criterion are ocular manifestations, more precisely iridocyclitis, uveitis or band-shaped keratitis. It has been pointed out repeatedly (5, 8, 9, 11, 27, 32), that these occur more often in children with RA than in adults which justifies their inclusion at least as a minor criterion. In the A.R.A. classification (6, 24) only

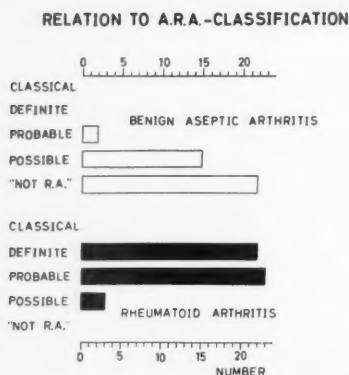


Fig. 6. Relation of the present diagnostic grouping to the A.R.A. classification.

iritis is mentioned and for some reason only among the criteria for the more vague group "possible RA". Further extra-articular systemic manifestations of the rheumatoid disease can hardly be considered as typical or specific enough to be included among the diagnostic criteria. As mentioned earlier, for the purpose of this investigation the diagnosis of RA was made if at least two major or one major and two minor criteria were present.

In analogy with the A.R.A. classification a list of exclusions more adapted for pediatric use has been established. Some of them need comment. There sometimes may be difficulties in the differential diagnosis of rheumatic fever, which has given rise to the suggestion of special diagnostic criteria (19, 23, 31). Transitional forms between rheumatic fever and rheumatoid arthritis are known, but as a rule a distinction is possible and the different diseases run quite different courses; the heart lesion being the decisive factor in the former and the joint affection in the latter. In this connection fulfilled accepted criteria for rheumatic fever must be re-

garded as an exclusion factor for the diagnosis of RA. Joint symptoms are frequent in Henoch-Schoenlein purpura, although admittedly rarely of a continuous duration of six weeks. Joint symptoms, as an extraintestinal manifestation of ulcerative colitis, have been observed to simulate the early stages of a RA and in one case they preceded the onset of the signs of colitis. A connection between urinary tract infection and polyarthritic symptoms has not been demonstrated in this material, but on the other hand this connection has been well established in adults (7) and must be kept in mind also regarding children. The other exclusion factors are in concordance with some of those listed in the A.R.A. classification.

It may be of interest to investigate how the diagnostic criteria presented here match the A.R.A. classification. This relation has been illustrated in Fig. 6. Among those labelled as BAA two cases fulfil the qualifications for "probable RA". In these two, symptoms were present from one knee joint for a little more than six weeks and from the other for about a month, followed by a rapid recovery without recurrences, the observation time being three years in the first case and six years in the second. Of the remainder 15 children with BAA satisfy the demands for "possible RA". This is not surprising in view of the short duration of the joint symptoms being required for inclusion in the "possible RA" group.

Of the cases considered to have RA according to the diagnostic scheme presented here none fulfils the criteria for "classical RA" in conformity to the A.R.A. classification. This is partly due to the imperfect notes on the occurrence of morn-

stiffness, the negative agglutination test in typical cases and the rarity of joint biopsies. As seen in Fig. 6 the present classification in rigorously lies between "definite" and "probable" RA". Three of the RA cases have not fulfilled more criteria than corresponding to "possible RA". In two of these there had been a long-lasting, recurrent monarthritis, one of them presenting a residual lesion at the follow-up examination. In the third case, which also ran a prolonged course, there was an interval exceeding three months between the two joints affected. There seems to be reason to regard these three cases as true RA.

The diagnostic scheme presented here being based on arbitrary conditions, shares the limitations of any attempt to construct diagnostic criteria in a similar way. It has, on the other hand, proved to be of practical value in this investigation, giving prognostic information at a relatively early stage. It has thus been possible to make a definite classification in close to half of the cases (48 per cent) within two months after the first hospital admission.

The cases labelled as RA in the present series correspond essentially to the descriptions of the clinical picture and characteristics of typical juvenile rheumatoid arthritis. Heredity for rheumatic diseases in its widest meaning was present in 25 per cent. In Sury's material true RA was present in nine per cent among close relatives (29), Edström gave the figure of 23 per cent (8), Sairanen 30 per cent (25). Johnson & Dodd (17) stated that the incidence of a family history of rheumatic disease was possibly as high as 30 per cent. The preponderance for girls of the magnitude two to one has also been a constant

finding in previous investigations. A peak incidence during the first three years of life has also been a regular observation. A preceding respiratory tract infection was found in the present material in 29 per cent. Similar figures have been given in several studies, but Johnson & Dodd (17) quoted references establishing the incidence as high as 37.5 to 57 per cent and were of the opinion that an upper respiratory infection was seen prominently at some time during the course in about 70 per cent of the cases. Trauma close to the affected joint, within one month before the onset, occurred in eight per cent. The analogous incidence given in earlier investigations has been of the same magnitude. A monarthritis is a common phenomenon early in the course. An initial monarticular localization for at least one month was present in 29 per cent, which is in concordance with corresponding figures given in the literature. In most of the present cases other joints were successively involved, but in six per cent of the whole material the disease remained monarticular. There is also a close conformity as to previous reports, of the typical distribution of joints being affected; the knee joints being most commonly involved, followed by wrists, ankles and fingers. Heart disease was an uncommon finding in the present material, probably mainly due to the fact that the material does not contain patients with a septic course, which disposes to systemic involvement. One further reason may be that ECG examinations have not been systematically made in each case. In Sury's series (29) two children died of a rheumatoid heart disease and seven per cent showed signs of cardiac damage at

the follow-up examination. Edström (8) reported seven patients with Still's disease all of whom had cardiac involvement and 22 per cent of the others had signs of a heart disease. The frequency of eye lesions is of special interest; iritis and uveitis occurred in 12 per cent in this investigation. Similar figures have been found by others (8, 29). A permanent visual impairment is not rare as a sequelae.

The concept that the prognosis in RA in children depends on how the material is selected seems justified. In a general pediatric ward a higher proportion of cases with a more limited course will be seen, whereas in a special institution for the treatment of RA, chronic and disabling cases will be overrepresented. The time of observation is of great importance. In the present investigation this comprises two to seven years, which is too short for a definite evaluation, but is probably enough to provide an approximation of the probable course. Of great importance also are the diagnostic criteria and if these are vague or too liberal, it will be difficult to compare one clinic's experience with that of another. Of the cases labelled as RA in this study most of them could be evaluated after three years' course. Fourteen per cent showed some degree of handicap. This is a relatively favorable figure as is evident from the prognostic review given in the introduction. This is most likely due to the fact that the patients were derived from pediatric departments. Even so the prognosis in general is somewhat better in children than in adults.

In the present investigation cases fulfilling the suggested criteria have been compared with another group indicated as benign aseptic arthritis. There is no

single symptom or finding placing a case in either category. The most important differences apply to the age of onset and the course. The RA cases commonly have their onset during the first five years of life; the BAA cases later. The course of the latter is shorter and does not leave any functional limitation. The preponderance for girls is the same in both groups and the distribution of joints being affected show similar trends, although a monoarticular course was more common among the BAA patients and the cervical spine was affected only in the RA cases. In both groups larger joints, especially the knees were most commonly involved early in the course. Ocular manifestations favor the diagnosis of RA and was included among the criteria. This was also the case concerning the positive agglutination reaction. Anemia was not uncommon among the RA children already within two months after the onset. A preceding infection was a common finding in both groups and to label the BAA cases as postinfectious as a distinguishing feature does not seem justified.

The many similarities between the RA and BAA group inevitably raises the question of whether they do not merely represent different courses of the same disease. This objection can hardly be contradicted, as long as the etiology of rheumatic diseases is unknown and no simple diagnostic test is available. As pointed out conventional, serological methods to demonstrate the presence of the rheumatoid factor are unreliable in children. Awaiting further advances in this field the diagnosis of RA has to be based on a combination of different criteria. This is essential, if different materials are



to be compared as to the results of various therapeutic trials. In a pediatric hospital cases are frequently seen with arthritic symptoms in connection with other diseases. The classification elaborated by the A.R.A. is not applicable to children without modifications. The criteria suggested here have been useful from a practical point of view, allowing a prognostic indication early in the course.

### Summary

Diagnostic criteria for rheumatoid arthritis adapted for the features of the disease in children have been suggested and used for a classification in a follow-up examination. This study comprised

87 cases of aseptic arthritis of at least three weeks duration hospitalized in pediatric departments in Stockholm during 1952-1957. Those not fulfilling the criteria were labelled benign aseptic arthritis. They were compared with the rheumatoid arthritis cases as to the early clinical picture and the prognosis. The entirely different course in the two groups indicates that the suggested classification is of value in the prognostic prediction in cases of aseptic arthritis in children.

### Acknowledgement

The valuable assistance of Mrs. Åsa Andersson and Miss Signe Wiberg is gratefully acknowledged.

### References

1. ANSELL, B. M. and BYWATERS, E. G. L.: Growth in Still's disease. *Ann Rheum Dis*, 15: 295, 1956.
2. — Prognosis in Still's disease. *Bull Rheum Dis*, 9: 189, 1959.
3. BARKIN, R. E.: The clinical course of juvenile rheumatoid arthritis. *Bull Rheum Dis*, 3: 19, 1952.
4. BILLE, B.: Kronisk polyartrit hos barn och dess guldbehandling. *Nord Med*, 37: 308, 1948.
5. BLEGVAD, O.: Iridocyclitis and disease of the joints in children. *Acta Opht*, 19: 219, 1941.
6. Diagnostic criteria for rheumatoid arthritis, 1958 revision by a committee of the American Rheumatism Association. *Ann Rheum Dis*, 18: 49, 1959.
7. DOMELJ, B., GIERTZ, G., OLHAGEN, B. and ROMANUS, R.: Genito-urinary focus in rheumatic disorder in the male. *Acta Chir Scand*, 115: 1, 1958.
8. EDSTRÖM, G.: Rheumatoid arthritis and Still's disease in children. A survey of 161 cases. *Arthritis Rheum*, 1: 497, 1958.
9. EDSTRÖM, G. and GEDDA, P. O.: Clinic and prognosis of rheumatoid arthritis in children. *Acta Rheum Scand*, 3: 129, 1957.
10. ELLMAN, P. and BALL, R. E.: "Rheumatoid disease" with joint and pulmonary manifestations. *Brit Med J*, 2: 816, 1948.
11. FRANCESCHETTI, A. and BAMATTER, F.: Augenveränderungen bei chronischer Polyarthritis im Kindesalter (Still'scher Formenkreis). *Ann Paediat*, 164: 60, 1945.
12. FYFE, W. M.: Rheumatoid arthritis in childhood. *Glasgow Med J*, 36: 102, 1955.
13. GAUCHAT, R. D. and MAY, C. D.: Early recognition of rheumatoid disease with comments on treatment. *Pediatrics*, 19: 672, 1957.
14. GROKOST, A. W., SNYDER, A. I. and RAGAN, C.: Some aspects of rheumatoid arthritis. *Bull Rheum Dis*, 8: 147, 1957.
15. ISDALE, I. C. and BYWATERS, E. G. L.: The rash of rheumatoid arthritis and Still's disease. *Quart J Med*, 25: 377, 1956.
16. JOHNSON, R. E. and HALL, A. P.: Rubella arthritis. Report of cases studied by latex tests. *New Engl J Med*, 258: 743, 1958.
17. JOHNSON, N. J. and DODD, KATHARINE: Juvenile rheumatoid arthritis. *Med Clin N Amer*, 39: 459, 1955.
18. JONES, T. D.: The diagnosis of rheumatic fever. *JAMA*, 126: 481, 1944.
19. KÖTTGEN, U. and CALLENSSEE, W.: Statistische Untersuchungen zum kindlichen Rheumatismus. *Der Rheumatismus*, Vol. 34. Dr Dietrich Steinkopff Verlag, Darmstadt, 1959.



20. McEWEN, C., ZIFF, M., CARMEL, P. DiTATA, D. and TANNER, M.: The relationship to rheumatoid arthritis of its so-called variants. *Arthritis Rheum*, 1: 481, 1958.
21. NORCROSS, B. M., LOCKIE, L. M. and McLEOD, C. C.: Juvenile rheumatoid arthritis. In "Progress in Arthritis", Ed. TALBOTT, J. H. and LOCKIE, L. M., Grune & Stratton, New York, 1958.
22. PICKARD, N. S.: Rheumatoid arthritis in children. A clinical study. *Arch Int Med*, 80: 771, 1947.
23. Report by the rheumatic fever working party of the Medical Research Council of Great Britain and the American Heart Association: Treatment of acute rheumatic fever in children. *Brit Med J*, 1: 555, 1955.
24. ROPES, M. W., BENNET, G. A., COBB, S., JACOX, R. and JESSAR, R. A.: Proposed diagnostic criteria for rheumatoid arthritis. Report of a study conducted by a committee of American Rheumatism Association. *J Chron Dis*, 5: 630, 1957.
25. SAIRANEN, E.: On rheumatoid arthritis in children. A clinico-roentgenological study. *Acta Rheum Scand, Suppl.* 2, 1958.
26. SCHLESINGER, B.: Rheumatoid arthritis in the young. *Brit Med J*, 2: 197, 1949.
27. SMILEY, W. K., MAY, E. and BYWATERS, E. G. L.: Ocular presentations of Still's disease and their treatment. *Ann Rheum Dis*, 16: 371, 1957.
28. STILL, G. F.: On a form of chronic joint disease in children. *Medicochirurgical transactions*, 80: 47, 1897.
29. SURY, B.: Rheumatoid Arthritis in Children. A Clinical Study. Munksgaard, Copenhagen, 1952. Thesis, University of Copenhagen.
30. TRONCY, R.: La maladie de Still chez l'enfant. Thèse, Faculté de Médecine et de Pharmacie de Lyon, 1950.
31. WALLGREN, A.: The diagnosis of rheumatic fever. *Ann Paediat Fenn*, 3: 548, 1957.
32. VESTERDAL, E. and SURY, B.: Iridocyclitis and bandshaped corneal opacity in juvenile rheumatoid arthritis. *Acta Ophth*, 28: 322, 1950.
33. WISSLER, H.: Der Rheumatismus im Kindesalter. Teil 2: Die chronische Polyarthritides Kindes. Verlag von Theodor Steinkopff, Dresden & Leipzig, 1942.
34. WISSLER, H.: Subsepsis allergica. *Helvet Paediat Acta*, 13: 405, 1958.
35. ZIFF, M.: The agglutination reaction in rheumatoid arthritis. *J Chron Dis*, 5: 644, 1957.
36. ZIFF, M., BROWN, P., LOSSPALLUTO, J., BADIN, J. and McEWEN, C.: Agglutination and inhibition by serum globulin in the sensitized sheep cell agglutination in rheumatoid arthritis. *Amer J Med*, 20: 500, 1956.

Submitted Dec. 20, 1960

Accepted Jan. 13, 1961

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## BOOK REVIEWS

**H. Jones: Reluctant Rebels. Re-education and Group Process in a Residential Community.**

Tavistock Publications, London 1960. 234 pages. Price 30s.

This book is based on an interesting pioneer experiment in residential therapy and re-education at "Woodmarch School", a community of about 40 disturbed boys aged ten to fourteen years, whose difficulties had arisen mainly from unfavourable home circumstances. The staff members cooperated to establish a secure, orderly but tolerant atmosphere. The boys were permitted to operate a system of partial self-government through various committees. Principles of sociometry and group psychotherapy were used in the rehabilitation process. It was possible in this way to get a better understanding and a better control of the proportion of social life that goes on beneath the surface of institutions of this kind. Lots of instructive examples are given from the everyday life at the school. Neighbourhood relations, staff problems and many other general problems usually connected with institutions of this kind are discussed. The book should be of great interest to everyone who works with institutions for mal-adjusted children.

*Tom Reinand, Göteborg*

**Edited by J. Chr. Siim. Human Toxoplasmosis. Munksgaard. Copenhagen, 1960. Price Dan. kr. 48:—.**

A conference on clinical aspects and diagnostic problems of toxoplasmosis in pediatrics was held in Copenhagen at the VIII International Congress of Pediatrics in 1956. Last year, the proceedings of this conference were reviewed and published in an elegant volume of 219 pages. In his introduction,

A. Sabin stresses the importance of quantitatively estimating the role of toxoplasmosis as a cause of actual disease in contradistinction to the accumulated information that is currently available on the high incidence of clinically inapparent infections. In six studies on congenital toxoplasmosis (by M. Lelong, F. Bamatter, H. Eichenwald and others) and in six on the acquired type (headed by the editor) the great variability of the clinical picture is amply exemplified. Differential diagnosis can be difficult, inclusion body disease can give the same clinical picture as full-blown congenital toxoplasmosis and the acquired disease is seldom pathognomonic. Isolation of the organism by biopsy and animal inoculation is very important and is stressed by all contributors. Serological tests are specific but only significantly rising titers indicate actual infection. (According to O. Thalhammer a low dye test titer and a negative skin test indicate a recent infection.) The editor gives the following indications for testing serum for toxoplasma antibodies: fever of unknown origin, lymphadenopathy, infectious mononucleosis (with negative Paul Bunnell test), relative lymphocytosis, encephalitis and serous meningitis, exanthema resembling typhoid rash, chorioretinitis and myocarditis. Following broad indications for biopsy and serological testing Siim has been able to show that 13 per cent of lymphadenopathies of unknown origin were caused by toxoplasma. Two papers deal with ocular involvement in toxoplasmosis, which may be a late manifestation of the congenital form but is also seen in the acquired disease. The treatment of toxoplasmosis is still in the tentative stage. A combination of chemotherapy and cortisone has probably been of some value in the acquired ocular form. Although the epidemiology is still obscure

it seems likely that animal toxoplasmosis is involved in the production of the disease in man. Much more work has to be done before we can prevent this puzzling disease.

*Rutger Lagercrantz, Stockholm*

**Helen B. Taussig: Congenital Malformations of the Heart. Second edition. Vol. I, General considerations.**

Harvard Univ. Press, 1960.

Volume I of the second edition of Dr. Taussig's book is designed for students and general practitioners. It corresponds to approximately 90 pages of the 1947 edition. The plan of the new volume is basically the same. All chapters have been revised, and a completely new 50-page part has been added on angiocardiology, aortography and cardiac catheterization. In the embryology section, Streeter's horizontal concept is used. The etiologic and genetic aspects of congenital heart disease are more fully discussed, but the principles of analysis of malformations, the changes in circulation at birth and the evaluation of the heart found at autopsy have not been brought up to date. In the chapter on methods of diagnosis, new material is added on aortic arch anomalies, the vascularity of the lungs, and the size of the pulmonary artery; the section on ECG is extended. It is stressed that angiocardiology and catheterization are only *aids* in diagnosis, to be used to clarify some specific point. Further, the dangers of these procedures are discussed at length. Comparatively little space is devoted to selective angiocardiology which is considered to give limited information and to be more dangerous. Less dilution of the dye and the risk of endocardial damage are mentioned as primary dangers. Many new illustrations have been added, mostly angiocardigrams which have been sharpened for didactic purposes.

Volume I of the revised edition of Dr. Taussig's work on congenital cardiac malformations represents a didactic survey for students and general practitioners.

*B. Ivarmark, Stockholm*

**Helen B. Taussig: Congenital Malformations of the Heart. Vol. II. Specific Malformations.**

Harvard Univ. Press, Cambridge 1960, 1049 pp. Price \$ 17.50.

The second volume of the new edition of Dr. Taussig's monograph corresponds to some 500 pages of the first edition. Consequently, much new material is included; many new chapters have been added, and large parts have been extended. The basic plan of the old edition is somewhat changed, the malformations being separated into four main groups, two according to the direction of the shunt and two dealing with the left and right side of the heart, respectively. As before, the treatise is focussed on the clinical picture with detailed information on physical findings with the addition of angiographic data. The illustrations comprise numerous radiographs, diagrams of the course of circulation and drawings of specimens. A new visual index gives a pictorial summary of signs and auscultatory, fluoroscopic and electrocardiographic findings. This index enables the reader to grasp the clinical essentials of the various syndromes at a glance and is superior to the old appendix correlating the diagnostic features.

New material in the group with venous-arterial shunt includes defective development of the right ventricle without VSD in which a discussion of Bernheim's paper would perhaps have been interesting. To this part is further added a valuable chapter on the Taussig-Bing heart, and another on arteriovenous aneurysms. The complete transpositions are more fully treated and in the truncus chapter the hemitruncus concept is added.

Part two treats malformations of the right side of the heart. New material in this category includes multiple peripheral pulmonary stenoses and a 39-page section on pulmonary hypertension.

In the left-to-right shunt part one finds a new section on aortic septal defect and a completely rewritten chapter on abnormal pulmonary venous connection, including

drainage into the inferior vena cava with sequestered lung ("scimitar syndrome"). The treatise on ASD now includes the atrio-ventricular defect.

In the fourth part new sections are found on vascular rings, coronary arteriovenous fistulae, fibroelastosis, glycogenosis, cor triatriatum and corrected transposition. The reviewer might miss the clinical correlations with detailed anatomical findings, but Dr. Taussig's monograph should still be a mine of information to cardiologists and pediatricians.

*G. Ivarmark, Stockholm*

**Dr. Med. Paul Heintzen: Quantitative Phonokardiographie am Beispiel der respiratorischen und pressorischen Schwankungen der Herzstätigkeit.** Georg Thieme Verlag, Stuttgart 1961. 187 pages, 101 figures. DM 26—.

After general discussion of the origin of different heart sounds, the author reports his own results from a study of the influence of respiration upon the phonocardiographic recording of the heart sounds. The effect of respiration upon hemodynamic conditions and the relation between phonocardiographic and hemodynamic changes during normal respiration and valsalva tests are discussed. A very complete bibliography on the subject is added.

*B. Strindberg, Stockholm*

**Th. Hellbrügge, J. Rutenfranz and O. Graf: Gesundheit und Leistungsfähigkeit im Kindes- und Jugendalter.**

Georg Thieme Verlag, Stuttgart, 1960. 293 pages. Price DM 34:—.

This monograph deals with certain problems of health and physical fitness in pediatrics. The 480 references give a good review especially of the German literature in this field. Several parts of the book are well known to the pediatrician, for instance the introductory chapters regarding mortality, morbidity, growth and development. Of greater interest are the parts that describe physical working capacity in different ages and its dependence upon hemodynamic, respiratory and muscular factors. Of value

also is the account of various methods for measuring the working capacity of an individual, in which the authors quote their own studies of the so-called "Leistungspulsindex" in a series of 940 subjects aged 8–25 years. In a chapter on recovery after work the pulse restitution is discussed in relation to the length of the anaerobic phase at the start of the test. The younger the individual the shorter is this phase and the quicker does the restitution after the termination of the work occur. The working and leisure conditions in school-children are compared with those in working adolescents. The authors review the degree in which national and international legislation and practice have taken into account the special physiology of the ages of growth.

*Yngve Larsson, Stockholm*

**F. Bertram and G. Michael: Internationales Biguanid-Symposium am 12. und 13. Mai 1960 in Aachen.**

Georg Thieme Verlag, Stuttgart, 1960. 167 pp. Price DM 16: 80.

The place of the sulfonyl-urea drugs in the treatment of diabetes is well established. As regards the more recently introduced biguanides the situation is more doubtful. The Aachen symposium in 1960 was arranged in order to throw more light on these substances. Among available biguanides the phenyl-aethyl compound, phenformin, is the most commonly used. In contrast to the sulfonyl-ureas its mode of action is extrapancreatic, otherwise unknown. The theory of stimulation of intracellular anaerobic glycolysis has not been satisfactorily proved, nor the related hypothesis of inhibition of respiratory enzymes. Of interest is the increase in lactate and pyruvate in blood serum during treatment with phenformin. In some cases a tendency to normoglycemic ketosis may make interruption of treatment necessary. The clinical experiences of the effect of phenformin and related biguanides are not unanimous, probably dependent on differences in the selection of cases for treatment. In older patients about 40–50 per cent seem to respond to the drug, in juvenile cases no more

than about one third. The experience of a pediatric clinic is reported by Rosenkranz, who found satisfactory results in 7 of 17 patients. This meant lower insulin requirement and stabilization of labile cases. Combined treatment with sulfonyl-ureas may in some patients be valuable. Gastrointestinal side effects may occur but do not seem to be a serious problem. One case of severe thrombopenia was reported by Koopmann, otherwise there have been no blood dyscrasias.

Yngve Larsson, Stockholm

**F. Gegesi Kiss and Gy. Szutrély: Herz- und Kreislaufstörungen im Säuglings- und Kindesalter.**

Verlag der Ungarischen Akademie der Wissenschaften. Budapest, 1960. 880 pages, 277 figures.

This book is the German translation of the second edition of a Hungarian publication which appeared in 1953. According to the preface, it is especially written for pediatricians and general practitioners. The authors base their statements on personal clinical experience, their own research work and current literature and especially quote their own publications, while most of the contributions by eminent foreign cardiologists such as Keith, Leatham, Nadas and Wood are overlooked. The very detailed theoretical discussions of certain concepts, for instance, cell-structure and metabolism of the myocardium, as well as diabetes mellitus, hypoglycemia and serum-disease, are given too

much space for a book meant for general practitioners and pediatricians. The congenital malformations of the heart, on the other hand, are too superficially treated. This chapter, even from a factual point of view, is not always beyond criticism. The statement that the growth in height is retarded in children with patent ductus arteriosus is too categoric and pertains only to more severe cases. Their opinion that bacterial endocarditis occurs very often in association with atrial septal defect is not in agreement with most authors. Among the diagnostic procedures the very valuable selective angiocardiology according to Jönsson and coworkers is not mentioned. The normal blood pressure in the pulmonary artery is given as 40/10 mm Hg which seems to be rather high. With regard to rheumatic fever the reviewer will only mention that the authors are of the opinion that the causative agent is a virus and that acute pericarditis often leads to constriction, a concept with which few authors will agree. The therapy of cardiac and circulatory failure is treated rather thoroughly and reflects the vast experience of the authors. The recommendations of restrictions with regard to sport and exercise after acute infectious diseases and cardiac operations seem rather severe. The book is not very attractive from a typographical point of view and is not easy to read. The figures are, however, rather good and satisfactorily instructive, even if not of the highest class.

L.-E. Carlgren, Gothenburg

## ANNOUNCEMENT

The Department of Pediatrics of the University of Buffalo School of Medicine has established a *research training program for persons planning a career in pediatric research and teaching*. Programs will be planned for each individual trainee for a period of at least two years with at least one year spent in study or research in a basic science department of the University of Buffalo School of Medicine, followed by a year of research

within the Department of Pediatrics. Qualifications include a minimum of one year pediatric residency. Stipends are \$6000 first year, \$6500 second year, plus \$300 per dependent up to three dependents. Funds are available for laboratory expenses and travel. Inquiries may be sent to Dr. Robert Gutrie, Children's Hospital, 219 Bryant Street, Buffalo 22, New York.

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From the Department of Metabolic Research (Head: Dr. Harry Boström), Wenner-Gren Institute, University of Stockholm, and the Pediatric Clinic (Head: Prof. John Lind), Karolinska Sjukhuset, Stockholm

## Studies on Ester Sulphates

### 12. Excretion of Sulphate Acceptors in Phenylketonuria

by HARRY BOSTRÖM and BO WENGLE

It is an established fact that many phenolic, indolic and steroid compounds are excreted in the urine of mammals. Before excretion, most of these compounds are converted to some extent into esters of either glucuronic or sulphuric acid. The enzymatic mechanism responsible for sulphate and glucuronide conjugation is nowadays known in some detail.

Sulphate conjugation has been shown to be a two-step reaction involving sulphate activation and sulphate transfer (12, 18), and the enzymes catalyzing these reactions are present in many tissues, e.g. liver, intestinal mucosa, cartilage and cornea. The enzymes involved in sulphate conjugation are water-soluble, and particle-free extracts containing sulphate-activating and sulphate-transferring enzymes have been prepared from various tissues. When a sulphate acceptor, e.g. a phenol or a steroid, is incubated in a medium containing an active tissue extract, ATP, inorganic sulphate and magnesium ions in a suitable buffer solution, the corresponding ester sulphate is formed *in*

*vitro* (11, 16, 17, 22). By adding  $S^{35}$ -labelled sulphate to such a system, the ester sulphate formed can easily be demonstrated by means of paper-chromatographic and electrophoretic methods, combined with autoradiography.

On the basis of these principles, a method was recently worked out in our laboratory for the demonstration of sulphate acceptors in human urine under normal and pathologic conditions (9). In the present investigation, this technique was applied in phenylketonuria—a condition known to be associated with characteristic disturbances in the metabolism of aromatic amino acids.

The main feature of this inborn error of metabolism is probably a defect in the enzyme system normally hydroxylating phenylalanine to tyrosine (13), which results in a raised excretion of phenylalanine (PA) and its derivatives phenylpyruvic acid (PPA),  $\beta$ -phenyl-L-lactic acid (PLA) and phenylacetic acid (PAA), the last-mentioned chiefly in the form of phenylacetyl glutamine (PAG) (14, 23).

In addition to the metabolites mentioned, some phenolic acids are excreted in

This study was supported by grants Nos. A. 2568 (M. & N.) and A. 4410 from the National Institutes of Health, Bethesda.



abnormal amounts. Thus the metabolites of tyrosine, *p*-hydroxyphenylpyruvic acid (*p*-HPPA), *p*-hydroxyphenyllactic acid (*p*-HPLA) and *p*-hydroxyphenylacetic acid (*p*-HPAA) are said to be excreted in raised quantities, as determined by paper chromatography (1, 7, 8, 21).

The most striking difference between the metabolism of phenolic acids in normal subjects and in phenylketonurics is, however, the hundredfold increased excretion of *o*-hydroxyphenylacetic acid (*o*-HPAA) in the urine of the latter (3). Although the mechanism of formation of this phenolic acid is uncertain, its precursor seems likely to be phenylpyruvic acid and not *o*-tyrosine, which has not yet been recovered from human urine (19).

A raised excretion of indolylactic and indolylacetic acids has also been demonstrated in phenylketonuria, thus indicating a deranged metabolism of tryptophan (2).

In 1951, Bickel introduced a low-phenylalanine diet in the therapy of affected children (5). It was found to normalize the excretion of phenylalanine and its metabolites, as well as that of *o*-HPAA and indolic compounds (3, 5, 15, 24). The excretion of tyrosine metabolites was, however, said to remain unaltered (7, 8).

In the present investigation, a comparison was made between the urinary "sulphate-acceptor pattern" of normal subjects and phenylketonurics. It disclosed that when urine specimens from phenylketonurics were incubated in the sulphurylating system, at least two conjugates were formed that could not be detected in normal urines treated in the same way. These findings will be reported and discussed in the following.

## Methods and Material

### *Sulphate-conjugation technique*

100  $\mu$ l of a carrier-free solution of  $S^{35}$ -labelled sulphate<sup>1</sup> (10 mC/ml) were evaporated to dryness in each of a series of test tubes. The test tubes were then placed in an ice-bath, and the following solutions added to each tube:

a. 50  $\mu$ l of a solution containing equal parts of 0.3 *M* potassium phosphate buffer, pH 6.8, 0.03 *M* potassium sulphate and 0.005 *M* magnesium chloride.

b. 10  $\mu$ l of 0.04 *M* ATP.

c. 40  $\mu$ l of a particle-free supernatant of rat-liver homogenate prepared according to Roy (16).

d. 10  $\mu$ l of human urine. If the urine was pretreated and extracted with ether, a portion of the extract corresponding to 50  $\mu$ l of urine was evaporated to dryness in the test tube before the  $S^{35}$ -labelled sulphate was added.

In each experimental series, two types of control experiment were run:

A. Complete system with enzyme solution and urine replaced by water.

B. Complete system with urine specimens replaced by water.

All test tubes containing the components listed above were then incubated in a water-bath at 37°C for 2 hrs.

### *Demonstration of ester sulphates*

Five  $\mu$ l samples of the incubated mixtures were subjected to paper electrophoresis and two-dimensional ascending paper chromatography on Whatman No. 1 filter paper. The following solvents were used:

I. Phenol-water (400 g/100 g). A beaker containing concentrated aqueous ammonia was placed in the chromatographic tank.

II. The upper phase of *n*-butanol-2 *N* ammonia (1/1 by vol.).

III. *n*-butanol-acetic acid-water (12/3, 5 by vol.).

<sup>1</sup> Obtained from the Radiochemical Centre, Amersham, England.



Of these three solvents, no. I was combined with either II or III. Solvent no. I was always used as first solvent.

Paper electrophoresis was performed in 0.075 M acetate buffer (pH 5.5) for 1 hr. at 30 or 40 V/cm.

The dried electropherograms and chromatograms were subjected to autoradiography on Gevaert Curix X-ray film, and exposed for one week. Gevaert G 150 was used as developer.

#### *Pretreatment of urine*

Addition of more than 10  $\mu$ l of urine to the enzyme system produced some inhibition of the sulphurylating activity. We therefore chose in some experiments to concentrate the urine specimens by freeze-drying and extracting the dry residue with ether, to take up aromatic and other organic compounds. These extracts were then free from salts, and could be added in amounts corresponding to 50–100  $\mu$ l of urine without inhibiting the system. In addition to untreated urine specimens, two types of urinary extract were incubated with the sulphurylating system, after evaporation of the organic solvent:

1. Ether extract of a freeze-dried urine specimen.

2. Ether extract of a neutralized and freeze-dried urine specimen, previously hydrolyzed in 1 N HCl for 60 minutes at 100°C.

All urines tested in the enzyme system were night specimens collected without the addition of any preservative. All subjects had been fasting since 8 p.m. on the preceding day. The samples were brought to the laboratory on the same day they were voided, and the experiments were started within a few hours of their arrival. Otherwise, the urine specimens were kept in deep-frozen condition until used.

#### *Chromatography of urinary amino acids*

Dealted urines were subjected to two-dimensional ascending paper chromatography on Whatman No. 1 paper, in the frames described by Datta, Dent & Harris (10). The following solvents were used:

- I. *n*-butanol-acetic acid-water (12/3/5 by vol.).

- II. Phenol-water-aqueous ammonia (400 g/100 g/2.5 ml).

The chromatograms were developed by dipping them into 0.2 per cent ninhydrin in acetone.

#### *Determination of phenylalanine in blood*

Determination of PA in blood specimens was performed by the method of Berry (4).

#### *Estimation of o-HPAA*

*o*-HPAA in urine specimens was estimated as follows. Urinary aliquots were chromatographed directly in amounts ranging from 20 to 100  $\mu$ l, depending on the amount of *o*-HPAA present. The solvent system used was *iso*-propyl alcohol–aqueous ammonia–water (20/1/2 by vol.), descending chromatography on Whatman No. 1 paper. The chromatograms were developed by spraying the papers with a dilute ethanolic solution of 2,6-dibromoquinone chlorimide, followed by aqueous ammonia. The intensity of the blue staining was compared with the colour of known amounts of *o*-HPAA chromatographed on the same paper. This spraying reagent was found to be more sensitive and specific to *o*-HPAA than diazotized sulph-anilic acid, and made it unnecessary to extract the substance from the urines before the chromatograms were run. Recovery of added amounts of *o*-HPAA to urine specimens was in the range of  $\pm 20\%$ .

#### *Preparation of reference substances*

The  $S^{35}$ -labelled ester sulphates of *o*- and *p*-HPAA were prepared from commercial preparations<sup>1</sup> of the latter by enzymatic sulphurylation *in vitro* in the rat-liver supernatant system, followed by isolation of the relevant esters by paper electrophoresis (autoradiographic indication), and subsequent elution and freeze-drying. The labelled material was stored at  $-20^\circ\text{C}$ .

The identity of the sulphate ester of *o*-HPAA formed was checked by identification

<sup>1</sup> Obtained from L. Light & Co., Colnbrook, Bucks., England.

of the split products, after acid hydrolysis in 1 N HCl for 1 hr. The liberated sulphate was shown to migrate in high-voltage electrophoresis at the same rate as a specimen of S<sup>35</sup>-labelled sulphate, and *o*-HPAA was identified in the aforementioned system for estimation of *o*-HPAA.

The ester sulphate of 5-(*p*-hydroxybenzyl)-hydantoin was prepared according to the same principle, from a specimen of the hydantoin obtained by synthesis from *p*-HPPA<sup>1</sup> and urea in the presence of hydrochloric acid (6).

In addition to these reference substances, several other ester sulphates of steroids, phenols and indoles have previously been produced by enzymatic sulphurylation in our laboratory, and their chromatographic and electrophoretic data have been established.

#### *Determination of creatinine*

Creatinine was determined by a modified Jaffe procedure (20).

#### **Case Material**

Eight cases of phenylketonuria were studied. In every case, the diagnosis was established biochemically, by demonstration of pathologically raised urinary excretion of PA, PPA and *o*-HPAA, and a high PA level in the blood. A summary of the relevant laboratory data is given in Table I. Cases 1-4 had not been given a low-PA diet. In Cases 5-8, this diet was tested with a varying degree of success. Urinary studies both before and during treatment could therefore be made in the latter group.

*Case 1.* A girl, b. 1942, sib of Case 4. She was severely mentally retarded, and was still unable to talk. Retarded development was first observed at 8 months of age.

*Case 2.* A boy, b. 1943. His I.Q. had been 30 since the first test at 8 years of age.

*Case 3.* A boy, twin brother of Case 2. Anthropologic studies, including blood typing, suggested monozygosity. His I.Q. had been 50 since the first test at 8 years of age. Retarded development was first observed in both twins at 6 months.

<sup>1</sup> Obtained from Dr. Theodor Schuchardt, GMBH & Co., München, Germany.

*Case 4.* A boy, b. 1944, sib of Case 1. He was severely mentally retarded, and was still unable to talk. Mental retardation was first noticed when he was 6 months old.

*Case 5.* A boy, b. April 15, 1956. Retarded development was first observed at 6 months of age. He had seizures, and his EEG was pathologic. Since Oct. 4, 1957 he had been given a low-PA diet. No marked mental improvement could be demonstrated after 2½ years' treatment. On the other hand, he ceased to have seizures, and his EEG became normal. In addition, his contact with the nursing staff improved considerably during treatment.

*Case 6.* A girl, b. Dec. 23, 1957. Her development was apparently normal up to at least 1 year of age. She sat without support at 7 months, and could say a few simple words before she was 1 year. When she was 1 year old, her mother noticed for the first time that the patient's urine had a peculiar smell. At 13 months, she started to walk, although somewhat unsteadily. One month later, she stopped walking, became less active, and seemed drowsy and tired. The diagnosis of phenylketonuria was confirmed at 18 months of age, and a low-PA diet was instituted. From being anxious and peevish, she became considerably more cheerful and active. At 20 months, she started to walk again, and to say a few words. Psychologic tests at 23 months of age showed somewhat retarded development, chiefly with respect to vocabulary.

*Case 7.* A boy, b. May 22, 1958. He was hospitalized at the age of 8½ months, on account of retarded development. He was unable to sit, and balanced his head unsteadily. He had seizures, and his EEG was pathologic. A low-PA diet was started at 9½ months. This resulted in a considerable improvement in his condition, including the EEG. His seizures stopped. His contact with his environment became much better, and he learnt to sit fairly steadily. He was able to stand without support before 17 months.

*Case 8.* A girl, b. Aug. 28, 1958. Asthmatic complaints started when she was a few

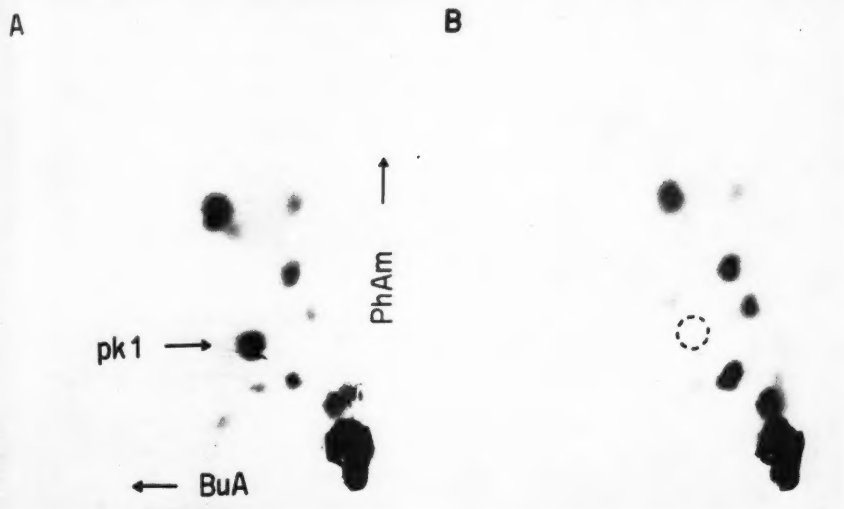


Fig. 1. Chromato-autoradiograms showing the "ester-sulphate pattern" of urine specimens *not pretreated* before incubation with the sulphurylating system. A. Urinary pattern of one untreated case of phenylketonuria (Case 6). B. Urinary pattern of one normal subject of the same age and sex as Case 6. The position of pk 1, as indicated on chromatogram A, corresponds to an empty area on chromatogram B (indicated by the dotted ring). PhAm and BuA represent solvents I and III, respectively (*cf.* text).

months old. Her urine had a strong, peculiar smell since the age of 4 months. She was unable to sit without support at 11 months, when a low-PA diet was instituted. At 16 months she could sit unsupported, but could not stand, even with support. Psychologic tests at 12 and 16 months showed no appreciable mental improvement. Her development seemed to be somewhat below the normal for a child half her age.

The controls consisted of 8 parents and 3 sibs of phenylketonurics, in addition to 20 healthy subjects, aged 3–53 years (members of the laboratory staff and their relatives), with no known history of phenylketonuria in their families.

## Results

### *Ester-sulphate pattern of normals*

Incubation of normal urine with the aforementioned sulphate-conjugating tech-

nique resulted in the formation of about 25  $S^{35}$ -labelled ester sulphates. They produced a characteristic pattern of spots on paper chromatography, or on paper electrophoresis in combination with autoradiography. Although this "ester-sulphate pattern" varied from case to case, its main features seemed to be constant (Fig. 1 B).

Incubation of ether extracts of normal urine specimens subjected to acid hydrolysis revealed a pattern differing in several respects from the original one. It contained more spots (ester sulphates) than originally present. On the other hand, certain ester sulphates formed when untreated urine specimens were incubated with the sulphurylating system could no longer be detected (Fig. 2 D).

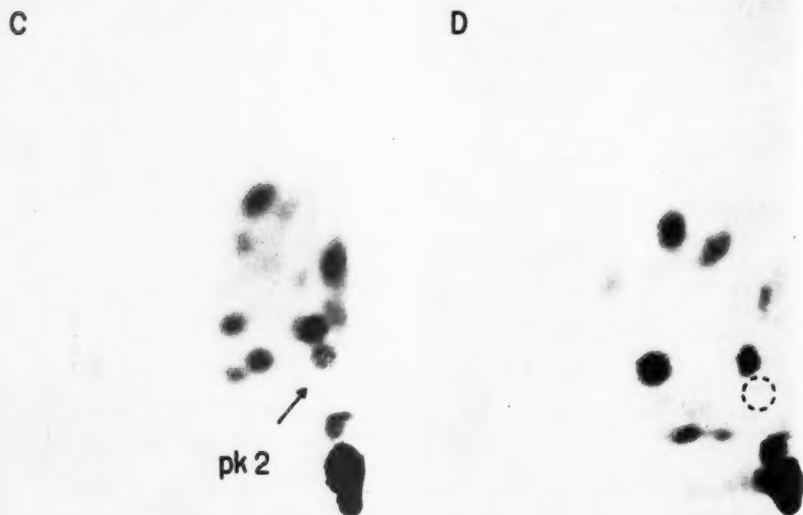


Fig. 2. Chromato-autoradiograms showing the "ester-sulphate pattern" of urine specimens *acidly hydrolyzed* before incubation with the sulphurylating system. C. Urinary pattern of one untreated case of phenylketonuria (Case 6). D. Urinary pattern of the same normal subject as in Fig. 1B. The position of pk 2, as indicated on chromatogram C, corresponds to an empty area on chromatogram D (indicated by the dotted ring). Solvent systems same as in Fig. 1.

#### *Ester-sulphate pattern of untreated phenylketonurics*

Urine specimens of seven untreated phenylketonuric patients, all excreting pathologically raised amounts of PPA and *o*-HPAA (Table 1), were sulphurylated as described. The sulphate acceptors of these pathologic specimens formed ester sulphates which produced patterns deviating characteristically and uniformly from those in normal subjects.

The pattern of urine specimens that were not pretreated showed, in every case, a new distinct spot, pk 1, on the autoradiograms (Figs. 1 A, 3 and 4).

The "ester-sulphate pattern" of acidly hydrolyzed phenylketonuric specimens revealed further peculiarities. In addition

to pk 1, another ester sulphate appeared, not present in normal patterns. This ester sulphate (pk 2) gave a well-marked spot on the chromato-autoradiograms when running the chromatograms in solvents I and III (Fig. 2 C). On the electropherograms, pk 2 moved relatively slowly toward the anode, which distinguished it fairly well from other labelled ester sulphates (Fig. 4).

#### *Ester-sulphate pattern of phenylketonurics given a low-phenylalanine diet*

Three phenylketonuric patients (Cases 5, 6 and 8) were considered to be adequately treated by a low-phenylalanine diet, in view of our biochemical findings (normal phenylalanine level in blood, nor-

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TABLE 1. *Summary of laboratory data in the 8 cases of phenylketonuria studied in the present paper.*

Case No.	Duration of treatment mths	Blood PA mg/100 ml	FeCl <sub>3</sub> and 2,4-dinitro-phenylhydrazine test <sup>1</sup>	Excretion of PA	Approx. amount of $\mu$ g <i>o</i> -HPAA/mg creatinine	pk 1	pk 2
1	0	35	+	+	170	+	+
2	0	25	+	+	150	+	+
3	0	30	+	+	100	+	+
4	0	40	+	+	110	+	+
5	0	30-40-50	+	+	pathol. amounts	?	?
5	29	< 2.5	—	—	< 5	—	traces
6	0	20	+	+	350	+	+
6	2½	< 2.5	—	—	< 5	—	+
7	0	25	+	+	75	+	+
7	13	?	traces	+	20	(+)	+
8	0	45	+	+	180	+	+
8	8	< 2.5	—	—	10	—	+

<sup>1</sup> A fresh aqueous solution of FeCl<sub>3</sub> and a saturated solution of 2,4-dinitro-phenylhydrazine in 2 *N* HCl were used as tests for the presence of PPA.

mal excretion of phenylalanine, PPA and *o*-HPAA: Table 1). Their urine specimens contained no sulphate acceptors able to form any detectable, abnormal ester sulphates when incubated unhydrolyzed in the sulphurylating system. Thus, pk 1 was lacking in the pattern. On the other hand, hydrolysis of the specimens showed that the sulphate acceptor giving rise to pk 2 was still excreted by two of the patients (Cases 6 and 8), despite several months' duration of treatment. The precursor of pk 2 seemed, in fact, to be excreted in the same amounts during treatment as before any dietary regime was started.

Urine specimens of the remaining patient (Case 5)—who had been treated for the longest period—gave rise to barely detectable amounts of pk 2, indicating that the excretion of its precursor had been practically normalized.

#### *Ester-sulphate pattern of relatives of phenylketonurics*

The urinary sulphate acceptors of eight parents (heterozygotes) and three unaffected sibs of phenylketonurics were also studied by the sulphate-conjugating technique. Neither pk 1 nor pk 2 could be detected in the "ester-sulphate pattern" of these persons, which therefore did not differ in any way from the normal pattern.

#### *Identification of pk 1*

The position of pk 1 on paper chromatograms (Fig. 1 A) and electropherograms (Figs. 3 and 4) was found to be characteristic for the ester sulphate of *o*-HPAA. Cross-matching experiments with reference substances in three independent chromatographic solvent systems (solvents I, II and III) and in one electrophoretic system (0.075 *M* acetate buffer,

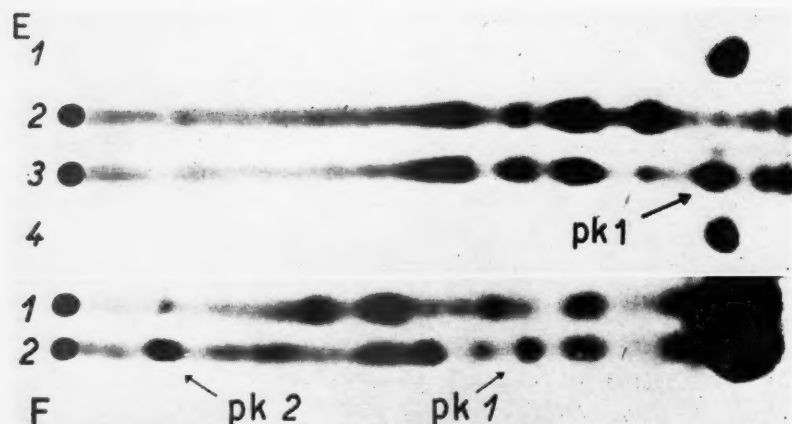


Fig. 3. Electrophero-autoradiogram showing the difference between the urinary "ester-sulphate pattern" of one normal (E 2) and one phenylketonuric boy (E 3). The urine specimens were *not pretreated* before incubation with the sulphurylating system. Starting points to the left in the picture. E 1 and 4, biosynthetically formed samples of the ester sulphate of *o*-HPAA. The position of pk 1 is indicated in the figure. Electrophoresis was performed in 0.075 M acetate buffer, pH 5.5 (40 V/cm).

pH 5.5, 30–40 V/cm) also indicated that pk 1 was identical with the ester sulphate of *o*-HPAA.

#### Identification of pk 2

It could further be shown that none of the hitherto known abnormal metabolites excreted in phenylketonuria could give rise to an ester sulphate with the properties of pk 2, when incubated in the sulphurylating system. In extraction experiments, it was found that the sulphate acceptor giving pk 2 could not be extracted with ether or ethyl acetate from the acidly hydrolyzed urines until they had been made alkaline with sodium bicarbonate. If, prior to hydrolysis, the urines were

Fig. 4. Electrophero-autoradiogram showing the patterns of the ester sulphates studied by chromatography in Fig. 2. Starting points to the left in the figure. F 1 corresponds to Fig. 2 D (normal). F 2 corresponds to Fig. 2 C (phenylketonuric). PK 1 and pk 2 are indicated in the figure. Electrophoresis was performed in 0.075 M acetate buffer, pH 5.5 (30 V/cm).

acidly extracted with ether or ethyl acetate, pk 2 could not be detected after incubation of the acidly hydrolyzed residues.

A mixture of *p*-HPAA and urea was hydrolyzed in the same way as urine, and incubated with the sulphurylating system. The hydantoin formed gave rise to an ester sulphate having the same properties as pk 2 on both chromatograms and electropherograms. Similar treatment of normal urines, after addition of certain amounts of *p*-HPAA, also revealed a labelled spot with the same characteristics as pk 2 on the autoradiograms of paper chromatograms and electropherograms.

During the hydrolysis of urine specimens of phenylketonurics, a compound was formed, which evidently consisted of 5-

Fig. 3.

Fig. 4.



(*p*-hydroxybenzyl)-hydantoin, its sulphuric acid ester being subsequently visualized on the autoradiograms as pk 2.

### Discussion

The enzymatic technique for demonstration of urinary sulphate acceptors worked out at this laboratory seems to possess high sensitivity. Thus, detailed ester-sulphate patterns containing about 25 compounds or more were revealed when 5  $\mu$ l samples of the incubating media (corresponding to 0.5  $\mu$ l of urine) were applied to chromatography. An increase in the sensitivity and specificity of the method was obtained by the slight modification introduced in the present study, *i.e.*, extraction of the urines before incubation. Another way of enhancing the possibility of detecting small amounts of certain compounds would be to raise the quantity of  $S^{35}$ -labelled sulphate added, or to expose the X-ray films for a longer period than one week.

Various solvents can be used for paper-chromatographic separation of the sulphated compounds. In the present study, phenol-water—which gave good spreading of the compounds—was always used as first solvent. *n*-butanol-acetic acid-water (III) was preferred as second solvent, because ester sulphates of phenolic acids of interest in phenylketonuria were much better separated in this system than in solvent II. Other solvent combinations might be more useful for the separation of other types of esterified compounds.

Hydrolysis of the urines prior to incubation increased the number of spots, and the overall pattern was greatly strengthened. A change in appearance of the patterns oc-

curred after hydrolysis, due to new compounds being available for sulphurylation, after splitting of acid-labile conjugates. In addition, certain compounds were evidently destroyed during acid treatment, since the patterns of untreated urines contained some ester sulphates that could not be detected after corresponding treatment of hydrolyzed urines.

The excretion of *o*-HPAA previously observed by other authors (3, 7) could easily be demonstrated by the present technique, since the most striking difference between the normal and phenylketonuric patterns was the appearance of the ester sulphate of this compound (pk 1). In agreement with earlier findings (3, 7), it could also be shown that the excretion of *o*-HPAA was normalized by a low-phenylalanine diet.

The studies on hydrolyzed urines from the patients did not disclose the excretion of any conjugated, pathologic sulphate acceptors. The new spot, pk 2—which actually appeared after the sulphurylation of hydrolyzed urines—was most probably identical with the ester sulphate of 5-(*p*-hydroxybenzyl)-hydantoin, a condensation product of *p*-HPPA and urea. It seems likely that the amount of pk 2 formed was correlated to the amounts of *p*-HPPA excreted, since the urinary urea concentration is certainly in excess for this condensation reaction.

Urine specimens of seven phenylketonurics gave rise to pk 2, despite the fact that some of them had been treated for a long period (2½–13 months). Pk 2 was, however, barely detectable in the ester-sulphate patterns of hydrolyzed urine specimens of Case 5 (treated for 29 months), indicating an almost normalized



excretion of *p*-HPPA. The difference between the strength of pk 2 in these patterns and in those of the other patients was great. Unfortunately, no data are available on the excretion of *p*-HPPA in Case 5 before a low-phenylalanine diet was instituted. There is, however, no reason to believe that the excretion of *p*-HPPA had been normal before treatment, since both the present study and earlier ones have shown that pathologic amounts of this acid are excreted in phenylketonuria.

It has also been claimed that the excretion of *p*-hydroxyphenyl derivatives remains unchanged during treatment of patients with a low phenylalanine diet (7, 8). The patient in question, who had been given this diet for 29 months, nevertheless excreted only small amounts of *p*-HPPA, which might indicate a slow normalization in the degradation of phenolic acids derived from tyrosine.

Of the cases of phenylketonuria discussed in this paper, Case 6 was the most remarkable from several aspects.—Firstly, this patient's development was obviously normal until at least one year of age, thus showing an unusually long lag period before symptoms of the disease appeared. Secondly, a rapid loss of already acquired abilities (e.g., walking, and saying a few words) occurred at the age of 13–14 months. Thirdly, a low-phenylalanine diet, which was not started until the age of 18 months, nevertheless restored the aforementioned abilities in a very short time.—The slow development of phenylketonuria could also be correlated to the biochemical findings in this patient, who most often showed comparatively low phenylalanine figures in blood, and sometimes even negative or only slightly posi-

tive tests for PPA in urine. It seems reasonable to suggest that this case represents a mild form of phenylketonuria. Moreover, it seems to demonstrate the value of dietary therapy, despite its late institution. The observations made in this patient thus seem to support the view that the critical factor in the dietary treatment of phenylketonuria is not only the age of the patient at the onset of treatment, but the actual duration of the manifestations as well.

The enzymatic technique applied in the present study of phenylketonuria did not reveal any previously unknown metabolite. On the other hand, the results obtained were in good agreement with our present knowledge of this disease. Consequently, it is suggested that this technique could be of value in the search for unknown sulphate acceptors in less well-characterized metabolic diseases.

### Summary

1. The pattern of sulphate acceptors present in the urine of phenylketonurics and control cases was visualized by chromato-autoradiographic methods, after treatment of urine specimens in a sulphurylating rat-liver system in the presence of  $S^{35}$ -labelled sulphate.

2. The sulphate of *o*-HPAA formed during this procedure from *o*-HPAA excreted in the urine of phenylketonurics could be demonstrated as a well-defined spot (pk 1) on the autoradiograms in all untreated cases.

3. Another sulphate acceptor, revealed after acid hydrolysis, was shown to be identical with the hydantoin formed from urea and *p*-HPPA during hydrolysis. The

concentration of the ester sulphate of this hydantoin (pk 2) probably reflects the amount of *p*-HPPA excreted in phenylketonuria. This compound did not vanish from the urine specimens of patients given a low-phenylalanine diet for periods of up to 13 months. It was, however, absent in one case treated for 29 months. The significance of these findings, as well as the possibilities of the method applied, are discussed.

### Acknowledgements

The authors' thanks are due to Professor J. Lind for his interest in our work, and for permission to perform this study in cases of phenylketonuria attending the Pediatric department, Karolinska Sjukhuset, Stockholm, as well as to Drs. E. Jacobsson, K.-M. Herrlin and A. Alvin, who kindly placed their cases at our disposal.

We are also grateful to Mrs. Kerstin Nordström, Mrs. Eva Lövgren, Mrs. Maria Déry, and Mrs. Ann-Britt Dahlberg for skilful assistance, and to Mrs. Erica Odelberg who revised the English.

### References

1. ARMSTRONG, M. D. and ROBINSON, K. S.: Abnormal metabolites in phenylketonuria. *Fed Proc*, 13: 175, 1954.
2. — On the excretion of indole derivatives in phenylketonuria. *Arch Biochem*, 52: 287, 1954.
3. ARMSTRONG, M. D., SHAW, K. N. F. and ROBINSON, K. S.: Studies on phenylketonuria. II. The excretion of *o*-hydroxyphenylacetic acid in phenylketonuria. *J Biol Chem*, 213: 797, 1955.
4. BERRY, H. K.: Paper chromatographic method for estimation of phenylalanine. *Proc Soc Exp Biol (N.Y.)*, 95: 71, 1957.
5. BICKEL, H., GERRARD, J. and HICKMANS, E. M.: The influence of phenylalanine intake on the chemistry and behaviour of a phenylketonuric child. *Acta Paediatr*, 43: 64, 1954.
6. BOSCO, R. J.: Unpublished observation. (Quoted by Boscott, R. J. and Cooke, W. T.: Ascorbic acid requirements and urinary excretion of *p*-hydroxyphenylacetic acid in steatorrhoea and macrocytic anaemia. *Quart J Med*, 23: 307, 1954.)
7. BOSCO, R. J. and BICKEL, H.: Detection of some new abnormal metabolites in the urine of phenylketonuria. *Scand J Clin Lab Invest*, 5: 380, 1953.
8. — Phenylalanine and tyrosine metabolism in patients with phenylketonuria. *Biochem J*, 56: 1, 1954.
9. BOSTRÖM, H. and VESTERMARK, A.: Studies on ester sulphates. 9. On the occurrence of sulphate acceptors in human urine. *Scand J Clin Lab Invest*, 12: 323, 1960.
10. LATA, S. P., DENT, C. E. and HARRIS, H.: An apparatus for the simultaneous production of many two-dimensional paper chromatograms. *Science*, 112: 621, 1950.
11. DE MEIO, R. H., WIZERKANIUK, M. and FABIANI, E.: Rôle of adenosinetriphosphate in the enzymatic synthesis of phenyl sulphate. *J Biol Chem*, 203: 257, 1953.
12. DE MEIO, R. H., WIZERKANIUK, M. and SCHREIBMAN, I.: Enzymatic system synthesizing sulphuric acid esters of phenols. *J Biol Chem*, 213: 439, 1955.
13. JERVIS, G. A.: Studies on phenylpyruvic oligophrenia. The position of the metabolic error. *J Biol Chem*, 169: 651, 1947.
14. — Excretion of phenylalanine and derivatives in phenylpyruvic oligophrenia. *Proc Soc Exp Biol (N.Y.)*, 75: 83, 1950.
15. KNOX, W. E. and EDWARDS, S. W.: Unpublished observation. (Quoted by Knox, W. E. and Hsia, D. Y.-Y.: Pathogenetic problems in phenylketonuria. *Amer J Med*, 22: 687, 1957.)
16. ROY, A. B.: The enzymatic synthesis of steroid sulphates. *Biochem J*, 63: 294, 1956.
17. SCHNEIDER, J. J. and LEWBART, M. L.: Enzymatic synthesis of steroid sulphates. *J Biol Chem*, 222: 787, 1956.
18. SEGAL, H. L.: Kinetic studies on the phenol-sulphate conjugating system of rat liver. *J Biol Chem*, 213: 161, 1955.
19. TASHIAN, R. E.: Phenylpyruvic acid as a possible precursor of *o*-hydroxyphenylacetic acid in man. *Science*, 129: 1553, 1959.
20. TAUSKY, H. H.: A procedure increasing the specificity of the Jaffe reaction for the determination of creatine and creatinine in urine and plasma. *Clin Chim Acta*, 1: 210, 1956.

21. WASSMUTH, M.: Über die Oligophrenia phenylpyruvica. Klinische, tierexperimentelle und biochemische, insbesondere papierchromatographische Untersuchungen. *Ann Univ Sarav (Med)*, 6: 184, 1958.
22. VESTERMARK, A. and BOSTRÖM, H.: Studies on ester sulphates. 10. On the sulphurylation of mono-, di-, and trihydric phenols. *Experientia*, 16: 408, 1960.
23. WOOLF, L. I.: Excretion of conjugated phenylacetic acid in phenylketonuria. *Biochem J*, 49: ix, 1951.
24. WOOLF, L. I., GRIFFITHS, R. and MONCRIEFF, A.: Treatment of phenylketonuria with a diet low in phenylalanine. *Brit Med J*, 1: 57, 1955.

Submitted Dec. 12, 1960

Accepted Jan. 26, 1961

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## Aplastic Anemia Treated with Anabolic Steroids and Corticosteroids<sup>1</sup>

by MARTIN SEIP

### Introduction

The treatment of aplastic anemia in childhood has hitherto mostly given discouraging results. Although remissions have occasionally been observed following corticosteroid therapy, splenectomy, or with supportive therapy only, a fatal outcome due to overwhelming infection or hemorrhage has been the rule. Of 40 patients treated at the Children's Hospital in Boston during a 20-year period only one survived after four years. Wolff (4) reported that not more than 3.3% were cured in a series of 334 patients (including both children and adults) suffering from aplastic anemia.

In 1959, Shahidi & Diamond (1, 2) reported promising results with a new therapeutic regimen, using testosterone 1-2 mg/kg body weight per day and small corticosteroid doses (triamcinolone 12 to 8 mg per day). A beneficial effect was obtained in seven of ten patients, while three died. In discussion following the paper by Shahidi & Diamond, other investigators presented similar experiences in a small number of patients (2).

In 1960, Shahidi & Diamond (3) re-

ported their results in 21 patients, 11 of whom were in remission and an additional three in incipient remission, while five were dead and two unimproved.

We have tried a similar, though not identical therapeutic regimen in three cases and have seen a convincing effect in two of them, while one desperately ill patient died a few days after the medication was started. We now use methandrostenedione (Dianabol, 'Ciba'), instead of the high testosterone doses, in order to reduce the incidence of side effects. This seems to be as effective, as judged by the results in our two cases.

### Case Reports

*Case 1.* A girl, born in 1947, developed signs of aplastic anemia when 7½ years old. Three years later, in April 1958, she was admitted to the Children's Department. A pancytopenia was present with hemoglobin 6.1 g%, r.b.c. 2.25 mill., w.b.c. 2800 with only 500 polymorphonuclears, thrombocytes 28,000, reticulocytes 1.2-2.0%. The bone marrow was markedly acellular. She was treated with corticosteroids and later with splenectomy, but only insignificant and transient improvement resulted.

In October 1959 her hemoglobin was between 8 and 9 g%, r.b.c. 2.5 mill., reticulocytes 1.8%, w.b.c. 2500 with 350 poly-

<sup>1</sup> Based on a paper read before Det norske Medicinske Selskab, Jan. 11, 1961.

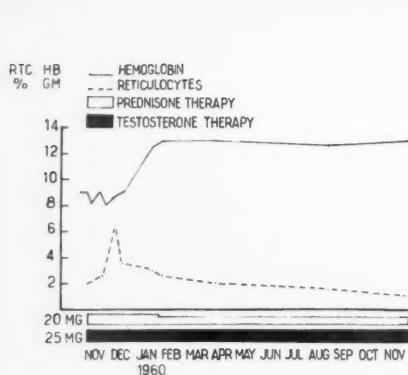


Fig. 1. Hemoglobin and reticulocytes, Case 1.

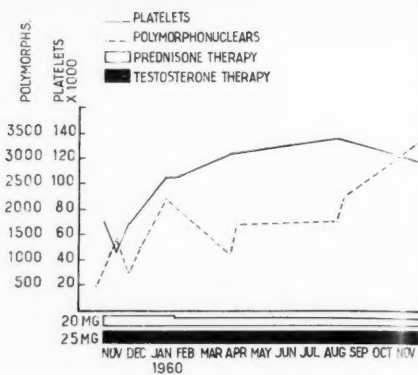


Fig. 2. Polymorphonuclear leucocytes and blood platelets, Case 1.

morphonuclears, blood platelets 40,000–50,000.

She was started on methyltestosterone 25 mg and prednisone 20 mg daily on November 3, 1959. One month later a reticulocytosis up to 6.5% developed (Fig. 1). Two and a half months after the medication was started her hemoglobin had risen to 12.5 g%, r.b.c. to 3.76 mill., w.b.c. to 5200 with normal differential count, platelets to more than 100,000. The bone marrow was much improved. The prednisone dosage was now reduced to 15 mg daily. Her blood picture has remained satisfactory (Figs. 1 and 2). Her general health is good, and there is no increased bleeding tendency. Rather severe acne and hypertrichosis developed on this therapeutic regimen. From November 28, 1960, methandrostenolone 10 mg daily has been given instead of methyltestosterone. Her blood values and whole clinical picture is as satisfactory on this form of therapy. Her acne is slowly improving.

**Case 2.** A boy, born 1954, the third of four siblings. The parents are healthy. A sister of the patient died at 7 years of age from Fanconi's anemia following a two year illness. The immediate cause of death was profuse bleeding due to thrombocytopenia. Treatment with corticosteroids, splenectomy and blood transfusions had been attempted to

no avail. The two other siblings are living and well.

The boy's anemia was discovered in March 1960, when he was almost 6 years old. One month later he was admitted to the Children's Department. His appearance was remarkably similar to his late sister's, and he had the same malformations, microcephaly with slight mental retardation (head circumference 49 cm) and pronounced epicanthal folds. His height was at the 10th percentile. He was pale and his skin was covered with scattered ecchymoses. A pancytopenia was disclosed with hemoglobin 7.7 g%, r.b.c. 2.89 mill., w.b.c. 3600, blood platelets 23,000 and reticulocytes 1.6%. Of 3600 leucocytes only 650 were polymorphonuclears. Bone marrow aspiration revealed a hypocellular specimen with depression of all elements. The erythropoiesis was somewhat better preserved than the myelopoiesis.

The patient was started on prednisone 40 mg daily and methyltestosterone 10 mg daily. Three weeks later the prednisone dosage was gradually reduced to 15 mg. After 7 weeks on this regimen the condition was almost unchanged, except for a moderate reticulocytosis and marked side effects, hirsutism, acne, flushing of the skin, obesity.

On May 31 methyltestosterone was therefore replaced by methandrostenolone

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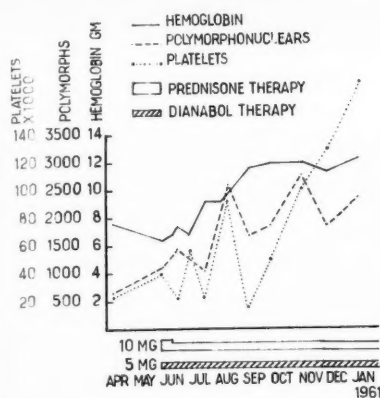


Fig. 3. Hemoglobin, polymorphonuclear leucocytes and blood platelets, Case 2.

(Dianabol), 5 mg daily. At the same time the prednisone dosage was reduced to 10 mg daily, twelve days later to 7.5 mg. He was then discharged from the hospital and has since then been treated as an out-patient.

He was seen again on July 16 and August 10. There was no obvious improvement in the blood picture, but his skin was more normal, his obesity less pronounced. On his next visit to the hospital on September 5 a remarkable improvement had occurred. He looked healthy. His hemoglobin had risen to 11.6 g%, r.b.c. to 3.8 mill., polymorphonuclear leucocytes to 1680 per  $\text{mm}^3$  (Fig. 3). The thrombocytes were still low (15,000).

The same therapeutic regimen has been continued and has resulted in further improvement. He is leading a normal life and has no increased bleeding tendency. His hemoglobin level has stabilized around 12 g%. His blood platelets have started to rise and now exceed 100,000 per  $\text{mm}^3$ .

### Discussion

We have been able to confirm the observations reported by Shahidi & Diamond that treatment with testosterone and corticosteroids may induce remission in some cases of aplastic anemia in child-

ren. In our two cases a definite improvement was noted between two and three months after the therapeutic regimen was started. A reticulocytosis was seen somewhat earlier, more so in one of the patients. This is in accordance with Shahidi & Diamond's experience. The beneficial effect on the erythropoiesis appeared earlier and was more pronounced than the effect on leucopoiesis and thrombocytopoiesis. Eventually significant increases in the number of polymorphonuclear leucocytes and platelets were also obtained. A remarkable increase in bone marrow cellularity occurred. The patients now feel well, have no abnormal bleeding tendency, and lead normal lives.

Our observations indicate that the same beneficial effect can be obtained whether methandrostenolone (Dianabol) or testosterone are combined with corticosteroids. The dose of methandrostenolone has been approximately 0.25 mg/kg body weight per day and the corticosteroid doses have been small. The marked side effects, which are inevitable when testosterone is given in the large doses recommended by Shahidi & Diamond (1-2 mg/kg body weight daily), can thereby be substantially reduced. Methandrostenolone and similar preparations have the anabolic properties of testosterone, while their androgenic effect is slight in the doses used. The risk of premature closure of the epiphyses is probably not important unless the testosterone therapy is continued for years, even so it should be considerably less with methandrostenolone. The observation, if confirmed in a larger series of patients, that the beneficial effect of androgens in some cases of aplastic anemia seems to be due to their anabolic rather

than their androgenic properties is of theoretic as well as clinical importance.

It should be stressed that the number of patients treated is still so small that further experience is necessary before a more detailed evaluation of these therapeutic regimens in aplastic anemia is possible. Aplastic anemia is not a single disease, and many patients will probably not respond to this new therapy. Whether the remissions induced will be permanent is as yet unknown, although Shahidi & Diamond followed their cases up to 3 years.

Whether and when the medication can be safely discontinued is unknown. Shahidi & Diamond discontinued the medication in six patients. One relapsed, while five were in relatively good remissions 4 to 14 months after the treatment was stopped. I would suppose that some patients, e.g. those with Fanconi's anemia, should be treated over a very long period

of time, possibly for the rest of their lives, while other patients can be tried without medication after about a year. Individualization is certainly necessary. A prolonged therapeutic regimen, if indicated, is easier to carry through with methandrostenolone than with testosterone.

It is remarkable that a beneficial effect has been observed in the Fanconi type of aplastic anemia, which has hitherto been invariably fatal.

### Summary

Two patients with aplastic anemia (one of them suffering from familial Fanconi anemia) are presented, in whom treatment with anabolic steroids and corticosteroids resulted in marked improvement. Our limited experience indicates that methandrostenolone produces much fewer side effects and may be as effective as testosterone in this condition.

### References

1. SHAHIDI, N. T. and DIAMOND, L. K.: Testosterone-induced remission in aplastic anemia. *Amer J Dis Child*, 98: 293-302, 1959.
2. — Testosterone-induced remission in aplastic anemia. *Amer J Dis Child*, 98: 474-476, 1959.
3. — Further observations on testosterone induced remission in aplastic anemia. *Amer J Dis Child*, 100: 753, 1960.
4. WOLFF, J. A.: Anemias caused by infections and toxins, idiopathic aplastic anemia and anemia caused by renal disease. *Pediat Clin N Amer*, 4: 469-480, 1957.

Submitted Jan. 18, 1961

Accepted Jan. 27, 1961

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## Conditioned Head Rotation Reflexes in Infants in the First Months of Life

by HANUŠ PAPOUŠEK

The experimental approach using conditioned reflexes has produced significant basic data on the early development of higher nervous system activity in infants. Food-seeking reflexes are particularly important in the first months of life, and upon their proper functioning depend health and life during the period of rapid growth in suckling young. For example, motor food-ingestion reflexes make their appearance by the third intrauterine month in the human fetus (Minkowski 1928), and are fully capable of function at birth.

Among motor food-ingestion reflexes, the conditioned sucking reflex has been thoroughly studied (2, 6, 8, 10, 19). Upon critical analysis we have observed several basic disadvantages of this method: it is not possible to employ conditioned sucking for estimation of more sensitive indicators of higher nervous activity, such as intensity of reaction and latent period; furthermore, from the methodological aspect, two contradictory trends are apparent with increasing age, regressive changes in the sucking reflex occurring at the same time as progressive development of higher nervous activity in infants (13, 14).

For these reasons we have attempted to employ turning of the head toward a source of food as the fundamental reaction for elaboration of conditioned reflexes in infants. In this communication data concerning the use of this new method in study of infantile higher nervous activity will be presented.

Head rotation reflexes are described in the literature as either the seeking component of suckling ("rooting reflex") or as orientational reactions. It is possible to evoke head rotation at birth by tapping the facial nerve at its outlet (3) or the facial muscles (1). Babkin distinguished this reflex movement from the "rooting reflex" and orientational head rotation, which have their centres in the diencephalon, and did not consider the latter reflexes as inborn. Bechterev & Ščelovanov (2) and Peiper (15), on the other hand, number them among the inborn basic reflexes present at birth. Stirnimann (22) reported that the seeking reflex is less constant than sucking activity in newborn infants up to 10-14 days of age, and concluded that this indicated regression in the biological significance of this reflex.

The food-seeking reflex is evoked primarily by gentle tactile stimulation of the



Fig. 1. Position of infant in the recording apparatus.

face in the vicinity of the corners of the mouth. The infant usually turns its head quickly toward the stimulating object and attempts to seize it in his mouth. During hunger, seeking movements may appear in the absence of any discernable stimulus (17, 18). Therefore, Popper (16) or Gamper & Untersteiner (7) number them among purposive movements. Rooting reflexes may also be called forth by temperature stimuli (5, 20, 21, 23), but not by olfactory stimuli (4, 19). According to Peiper (15), natural conditioned reflexes associated with obtaining the bottle develop during the first month of infancy. With suitable positioning seeking movements can be evoked in all three axes—vertical, sagittal and transversal. After satiation, both conditioned and unconditioned food-seeking activity is suppressed.

Head rotational reflexes in the sense of a conditioned orientational reaction were studied by Kasatkin *et al.* (9), who described objective registration of head rotation employing fixation of threads to each temple, leading over pulleys to penwriters, one recording movements to the left, the other to the right.

Our studies on conditioned food-seeking head rotation were begun in 1956, and an original method was worked out and used throughout these studies (13).

### Methods

**Recording.** The infant lay in a crib fitted with an elastic pad, which allowed registration of overall activity as well as partially immobilizing the infant so as to eliminate disturbing activity (Fig. 1). During movements of the infant the pad oscillated, and

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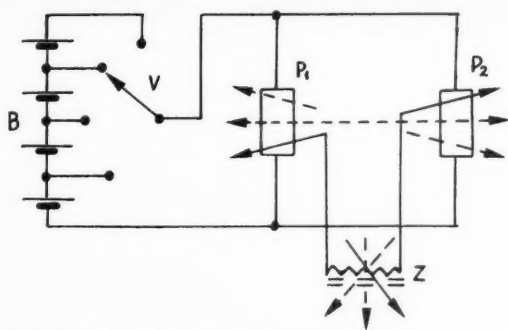


Fig. 2. Diagram of mechanical and electrical registration system (design J. Schenzer). B = source of direct current 8 V. P 1, P 2 = potentiometers  $50 \Omega/2$  W. V = potential selector (2-8 V). Z = electromagnetic penwriter  $400 \Omega$ .

the oscillation, transmitted through a pneumatic system, was recorded kymographically as an actogram. In order to compare actograms of children of different body weights, special calibration of the kymograph is necessary (Papoušek 1956). Pneumatic registration of respiration was usually carried out simultaneously.

The infant's head was placed in an independent padded cradle, which rotated about the horizontal axis with rotation of the head. Two potentiometers, joined as shown in Fig. 2, were placed in this axis with a battery or a rectified transformer as voltage source. Changes in potential occurring with rotation of the head in this axis were registered with a high resistance electric penwriter, connected between the slides of the potentiometers. The sensitivity of the recording system was given by the potential applied to the potentiometer circuit (2-8 V). In our system the kymograph penwriter gave deviations of 1 or 2 cm with head rotation of  $90^\circ$  (Fig. 3).

An important factor was the placing of the axis about which the head cradle rotates. A screw, fixing the cradle to the axis, could be moved vertically, thereby enabling the cradle to be balanced so that even a newborn infant had no difficulty holding its head in the central, neutral position. On an ordinary crib surface an infant does not hold its head in this position during the first few

months of life, but lies with the head turned to one side or the other, according to its intrauterine position.

*Conduct of experiments.* An acoustic stimulus—the sound of an electric bell or buzzer—was used as the conditioning stimulus. This was oriented centrally above the crib. Milk was offered from one side or the other as the unconditioned stimulus. The milk was given by a nurse, sitting concealed at the infant's head, and offered in such a way that the infant could not see the nipple without turning its head.

In preliminary observations it was ascertained that the sounds of bell or buzzer were indifferent with respect to this experimental situation. A conditioned reflex to the bell was then elaborated, offering milk from the left. The bell rang for a maximum of 10 seconds before milk was given. If the infant turned its head to the left, it received the nipple immediately, and the bell was turned off. If the infant did not seek and receive the milk within 10 seconds, the nurse first touched the left corner of the mouth with the nipple and, if this tactile stimulus was ineffective, turned the infant's head, and placed the nipple in its mouth, after which the bell was turned off.

The experiment was thus arranged so that the more rapidly the infant reacted correctly, the sooner he received the milk. As long as he did not carry out the correct

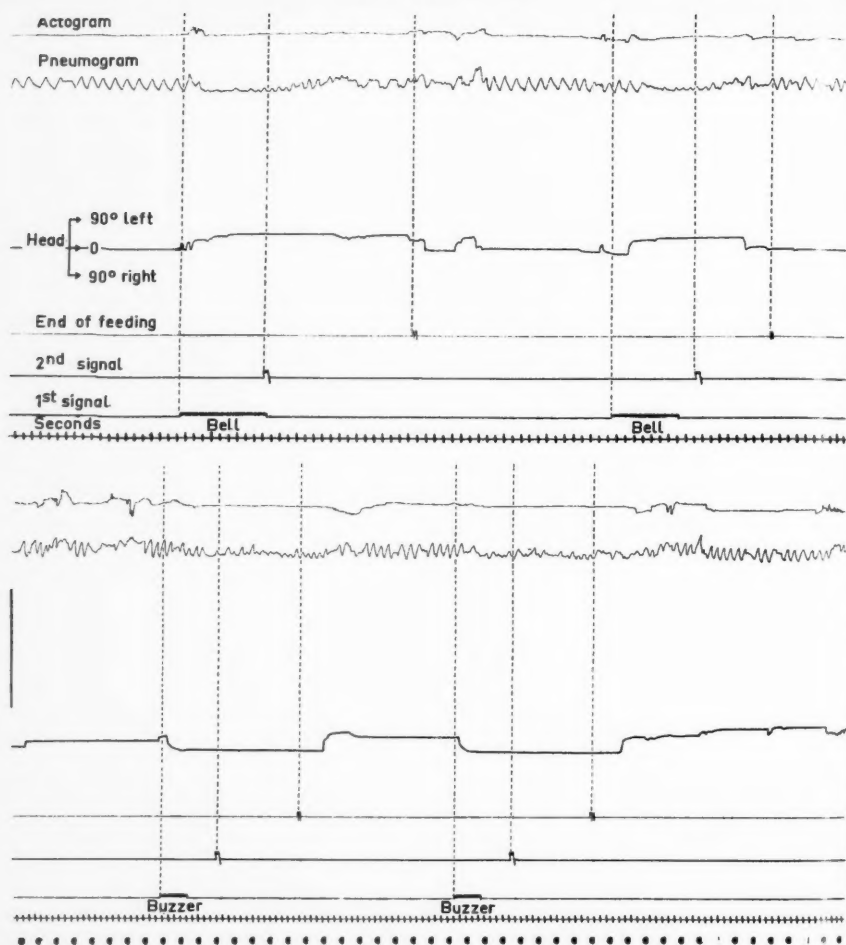


Fig. 3. Example of kymographic record of conditioning of head rotation in 5-month-old infant T.S. From above: actogram, pneumogram, rotation of head, end of feeding, beginning of feeding, conditioned signal, time signal (seconds). The 1<sup>st</sup> part shows the positive reactions to the bell (to the left), the 2<sup>nd</sup> part the reactions to the buzzer (to the right).

movement, he received nothing to drink. In this way the practical significance of a correct response was emphasized and probably explains the gradual shortening of the latent period with adaptation to experimental conditions.

When conditioning was so firmly established that the infant carried out 5 successive

correct responses, reinforcement of the bell stimulus was stopped, and the extinction of the conditioned reflex studied. Again, when the infant did not react positively to the bell stimulus 5 successive times, the conditioning was renewed, and differentiation of two positive stimuli studied. A new stimulus—a buzzer—was introduced and reinforced

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TABLE 1. *Number of stimuli required for elaboration or extinction of conditioned head rotation.*

Infant N°	Age at beginning of investigation (days)	Number of stimuli	
		a elaboration	b extinction
33	3	455	—
32	17	193	29
31	19	275	24
44	20	73	26
45	20	57	60
43	21	169	35
50	55	26	65
46	56	95	14
41	56	95	46
48	56	75	75
42	56	45	12
49	56	19	—
34	85	68	30
39	85	58	25
37	85	25	37
40	86	55	29
35	88	62	18
36	89	44	17
30	131	41	12
28	140	35	60
47	140	26	34
29	143	40	22
27	146	17	50

by administration of milk from the right side. The criterion of elaboration of differentiation was taken as successful responses to 6 successive stimuli (3 bell and 3 buzzer in random order). After accomplishing this differentiation, the signals were reversed, with bell for right side reinforcement and buzzer for the left.

One session was limited to 10 conditioned stimuli as a rule. Care was taken not to have intervals between stimuli the same. A session, once daily, lasted 10–15 minutes. It usually took place at the forenoon feeding time (10–11 o'clock), and before it the infant slept.

In our experience an infant at rest does not move its head more than 30° from the neutral supine position. Rotation of the head 30°–60° is taken as a weak positive response and more than 60° as a strong positive

response. These angles can be defined with suitably placed contacts or with various types of electrical signals.

*Selection of infants.* Care was taken that reproducibility of experimental conditions was not interfered with by pathological factors affecting head rotation (postnatal hemorrhage into the sternocleidomastoid, asymmetry of cervical musculature, deformity of the skull, neurological abnormalities etc). The physiological tendency of the newborn to turn its head on one side, usually the right, did not prove a serious hindrance, since in this apparatus, as already mentioned, even a newborn infant had no difficulty holding its head in the neutral position.

From 1956 to 1958, 23 healthy fullterm infants were studied. Throughout the period of study the infants remained under the care of personnel in the research ward for healthy

TABLE 2. *Number of stimuli required for elaboration and double reversal of differentiation between two positive stimuli.*

Infant N <sup>o</sup>	Age at beginning of investigation (days)	Number of stimuli		
		a differentiation	b 1 <sup>st</sup> reversal	c 2 <sup>nd</sup> reversal
44	43	31	73	49
43	61	211	44	20
45	62	163	93	49
32	63	149	25	—
42	67	151	23	39
50	76	123	92	20
41	88	56	26	29
48	90	129	27	35
31	92	82	—	—
40	103	106	42	31
39	104	123	59	33
36	108	85	50	18
37	112	88	73	44
35	113	126	—	—
34	119	127	42	40
47	153	51	84	112
28	156	51	—	—
30	165	7	—	—
29	174	47	—	—
27	159	discharged unsuccessful up to the age of 167 days		
46	80			

infants (84,6 days on the average). In all, a total of 13,422 stimuli in 1457 sessions were employed. In one infant elaboration of conditioned reflexes was begun at 3 days of age. The remaining infants fell into 4 narrow age groups at the beginning of the study (Table 1).

### Results

Table 1. (a) shows the rapidity of elaboration of conditioned head rotation, as measured by the number of stimuli necessary to achieve the given criterion. In infant 33 a stronger stimulus was employed, and experimental sessions were more frequent in order to achieve maximal rapidity of conditioning, so that this infant is not comparable to the rest of the group. The first weak positive response was observed on the 5<sup>th</sup> day of age, after 58 stimuli, but not until the 8<sup>th</sup> day and 83

stimuli did conditioned reflex begin to appear regularly. The criterion of 5 successive positive responses was attained by this infant on the 32<sup>nd</sup> day of life, after 455 conditioning stimuli. In the remaining infants there was a statistically significant direct relationship between age and rapidity of conditioning ( $P < 0,01$ ). Individual differences, however, appeared in every age group.

The extinction of conditioned reflexes occurred more rapidly than their elaboration, but the rapidity of extinction was related neither to age, nor rapidity of original conditioning, and individual differences here were not marked. The number of non-reinforced stimuli preceeding extinction (by the criterion described above) are also presented in Table I (b).

Table 2 (a) presents data concerning rapidity of differentiation of two positive stimuli in individual infants. The rate was taken as the number of new stimuli (buzzer) preceeding successful differentiation.

In almost all children differentiation was learned without difficulty. Only infant 46 failed to achieve differentiation even after 50 days. In this infant one had the impression that the learning task was simply too difficult, he reacted more frequently with restlessness during sessions, gave chaotic responses and differentiation remained very weak and erratic. Infant 27 was inadvertently discharged from the ward before achieving this phase of conditioning.

As with primary elaboration of conditioning, a statistically significant dependence upon age was apparent ( $0.02 > P > 0.01$ ). With increasing age a negative selection of infants began to become of significance, since infants who were the slowest to elaborate primary conditioning were thereby oldest at the beginning of differentiation studies. We did not, however, find any correlation between rapidity of differentiation and rapidity of elaboration or extinction of primary conditioning. Individual differences were also apparent in the rapidity of elaborating conditioned reflexes to a new stimulus (buzzer) and in the influence of the new stimulus upon the level of conditioned response to the original stimulus—bell. In addition there appeared interesting individual differences in some phenomena accompanying conditioning (total motor activity, respiration, vocal and facial reactions), which require further study.

Table 2 (b) shows the rate at which in-

fants achieved differentiation when the two positive signals were reversed—i.e. when they signaled reinforcement with milk from the opposite side. The rate is expressed as the number of buzzer signals preceeding achievement of the criterion of the differentiation. The number of infants gradually decreased, since it was not possible to keep them all on the ward for a sufficiently long period.

When the infants are arranged by age, it is apparent that infants in whom the preliminary phase lasted longer predominate. This negative selection was probably the reason why no significant relationship to age was demonstrated in this group. On the contrary there was a tendency for older infants to elaborate this process more slowly. With the exception of two infants (44 and 47), the reversal of differentiation occurred more rapidly than the original process of differentiation.

The second reversal of differentiation, in which both signals reverted to their original meaning, was also studied. As can be seen in Table 2 (c), the second reversal of differentiation signals was followed by rather faster learning than during the first reversal or original differentiation. Again, no statistically meaningful relation was found to age or to previous phases of the experimental situation.

This method has enabled measurement of latency and intensity of head rotation responses with relative ease. The latent period seemed characteristic for individual infants. It tended to decrease gradually with elaboration of the conditioned reflex and with age, but in certain infants was always longer than in others. In the course of differentiation, the latent period first lengthened, particularly in younger in-



TABLE 3. *Individual differences in latency of response.*

a = average latency (seconds) of first 10 correct responses  
 b = " " " " of middle 10 " "  
 c = " " " " of last 10 " "

Infant N <sup>o</sup>	elaboration			Average latency during differentiation			1 <sup>st</sup> reversal		
	a	b	c	a	b	c	a	b	c
34	2.96	4.65	3.53	2.70	—	2.65	1.20	1.05	0.75
40	3.07	3.52	1.67	1.67	2.75	2.42	1.90	1.90	2.60
42	3.53	3.12	1.82	3.40	4.80	—	2.96	3.26	3.84
39	3.87	3.17	4.02	2.20	1.70	0.68	3.32	2.52	1.37
50	4.90	—	5.07	2.14	4.50	2.48	2.55	2.85	2.20
47	5.33	—	4.66	2.76	3.03	3.02	3.47	3.15	3.31
45	5.44	6.43	6.22	3.36	5.36	2.96	2.18	2.22	1.85
44	5.80	5.91	4.96	4.36	4.41	3.36	3.39	3.41	3.27

fants, and then again decreased. In addition, the latent period was found to reflect temporary influences sensitively—the degree of hunger, various changes in the dynamic stereotype and experimental conditions, the course of the previous response etc.

A detailed analysis of these associated subtle indicators of the functional state of higher nervous activity deserves a separate communication. Here, for illustration only, changes in individual latent periods and their changes in the course of investigation are shown in Table 3.

In general, the remarks above apply to the intensity of response. In our experience, however, intensity of reaction is a less sensitive indicator than latent period. In this case, we also observed dependence upon age, the degree of elaboration of conditioning, and individual characteristics. In younger infants, unclear reactions appeared first, followed by weak responses, which gradually strengthened. In older infants reactions were sometimes very striking, even after only few reinforce-

ments. During extinction of conditioned reflexes the intensity of response gradually decreased. Intensity often decreased temporarily during differentiation and reversed differentiation.

In addition to intensity and latency of reactions, it was also possible to estimate the rapidity with which the infant turned his head. It has been a frequent observation that a strong, rapid reaction had a short latent period and conversely.

### Discussion

These studies have confirmed that reflex rotation of the head toward a source of food is advantageous methodologically for the investigation of conditioned food-seeking reflexes even in newborn infants. In our results, the influence of age upon elaboration of conditioned reflexes has been found to be quite constant, unlike results using the sucking reflex, which simplifies analysis of developmental processes in higher nervous activity.

It has been found that data on basic indices of higher nervous functions can be obtained, even in the first half year of life. A sufficiently well-established conditioned reflex can be elaborated by the beginning of the 2<sup>nd</sup> month, differentiation between two positive stimuli by the 3<sup>rd</sup> month and reversal of differentiation by the 4<sup>th</sup> month. The latter was even achieved, in one infant, in the 3<sup>rd</sup> month of life.

In addition to data on the rapidity of the conditioning process, this method has also permitted study of more subtle indicators of cortical function, such as the latency and intensity of conditioned responses. This experimental approach has the added advantage that the infant receives milk to drink only when it carries out the correct motor reactions, which is a powerful impulse toward shortening of the latent period of response with adaptation to the experimental situation. We believe this experimental approach to have more than purely methodological interest, for it has already yielded interesting data on latency of motor responses at this age, which as yet have not been described. Observations on the relatively complex differentiation of which 3 or 4 month old infants are capable have not as yet been reported.

The circumstance of elaboration of two analogous conditioned reflexes—rotation of the head to the left or right—opens important new methodological possibilities. First one can study elaboration of two reinforced stimuli. This is superior to using differentiation between a reinforced and non-reinforced stimulus in that it does not evoke the marked restlessness often produced by, for example, frustration of the sucking reflex when milk is not forth-

coming. It also permits comparison of two symmetrical reflexes under ideally comparable conditions, observation of the influence of the mode of reinforcement, etc.

This method also opens the way to a study, not of pure motor responses alone, but of the development of purposeful adaptive activity as a whole. Using the experimental situation described, reflex rotation of the head may be given the meaning of a purposeful food seeking response, an orientational reaction, or even a defense reaction. One can study how a simple basic movement, easily registered, develops into a complex adaptational response, to which an infant imparts various meanings with various stimuli. To our knowledge, analysis of purposeful activity at such an early age does not appear in the literature. For that matter it has not been suggested that 3-4 month old infants are even capable of such complex reactions as appeared in these observations. This is probably because heretofore coordination of activity in limbs, particularly the upper, has usually been studied. However, if one studies head movements which according to the principle of cephalocaudal development of motor activity mature sooner than movements of extremities, differentiation of motor reactions can be detected earlier than has been hitherto supposed.

The results of the investigation using conditioned head rotation may be summarized as follows:

- 1) Conditioned rotation of the head toward a source of food may be used for the study of conditioned reflexes in infants the first few months of life. Responses are obvious, and easily registered

kymographically. Analysis of records is simple. Registration of head rotation and total motor activity may be carried out simultaneously.

2) It is possible to elaborate a sufficiently stable conditioned reflex, on the basis of unconditioned head turning, by 5-6 weeks of age. Weak and unstable conditioned responses were observed in one infant in the 2<sup>nd</sup> week of age.

3) It is possible to elaborate differentiation between two positive stimuli. The infant thus elaborates two symmetrical hetero-lateral but entirely analogous reflexes. One stimulus is reinforced by giving milk from the left, the other from the right. Such differentiation was achieved in one infant the 2<sup>nd</sup> month, but in the majority it appears during the 3<sup>rd</sup> month. By the 6<sup>th</sup> month it was possible to finish two reversals of differentiation of two positive stimuli. In one infant this was accomplished by the end of the 2<sup>nd</sup> month.

4) The original elaboration of a conditioned reflex and differentiation of two positive stimuli is dependent upon age, and this age correlation is statistically significant—the older the infant, the more rapid conditioning or differentiation. Extinction of conditioning and renewal of the conditioned response, on the other hand, are not related to age. No relationship with age was apparent in reversal of differentiation, since here a negative selection of individual infants operated (those infants slowest at achieving the preliminary stages of conditioning, reached this phase at the oldest ages).

5) In addition to data on rapidity of conditioning, this method has also yielded more subtle indications of higher nervous activity—latency and intensity of condi-

tioned responses. Observations of the latency of motor reactions in very young infants were obtained. Latency decreases as conditioning progresses, more rapidly in older infants. Changes in latency were considered to be manifestations of cortical adaptation to the experimental situation, and the situation was designed to demonstrate this, since the rapidity with which the infant carried out the correct response determined the rapidity with which it obtained milk to drink.

6) Employment of conditioned head rotation, as described here, is suitable for other than the study of conditioned food-seeking reflexes. Since it is possible to elaborate two entirely analogous symmetrical reflexes under ideally comparable conditions, it permits comparison of the influence of modification of the conditioning stimulus, unconditioned reinforcement and other experimental conditions.

This experimental approach is also promising for the study of purposive activity. Head rotation is singularly suited to the study of how a basic responsive movement becomes modified under various conditions. Head rotation may function in various circumstances as a component of food-seeking activity, orientation or defense reactions. A further advantage lies in the fact that purposive head movements by the principle of cephalocaudal development, appear earlier than those of extremities, and are also easier to register.

### Summary

A new experimental system for study of higher nervous function has been tested with 23 normal fullterm infants. This method employs conditioned rotation of

the head recorded electro-mechanically. As compared with conditioned sucking studies, this method has been found advantageous from several points of view.

New data on the development and coordination of motor responses in young infants have been obtained. A sufficiently stable conditioned reflex may be achieved at 4-6 weeks of age, differentiation of two positive stimuli at 3 months and double reversal of this differentiation in the 4<sup>th</sup> month. The experimental approach described permits study and comparison of two analogous symmetrical heterolateral reflexes and simple analysis of intensity

and latency of response as sensitive indicators of cortical functions. It also opens new possibilities for the study of early purposive motor reactions. Since rotation of the head may take on various meaning—food-seeking, orientation, defense—it is particularly well suited to the study of ontogenetic change of one simple motor act into a complex adaptative mechanism.

### Acknowledgements

The author wishes to thank Sisters Vlasta Lapáčeková and Jarmila Melicharová for their devoted and skillful assistance in both nursing care and research investigation.

### References

1. BABKIN, P. S.: Ščečno-vrašatel'nyj refleks golovy u grudnykh detej (Facial rooting reflex in infants). *Ž. Nevropat Psichiat Im Korsakova*, 9: 692, 1953.
2. BECHTEREV, V. M. and ŠCELOVANOV, N. M.: K obosnoveniju genetičeskoj refleksologii (The motivation of genetic reflexology). *Novoje v refleksologii*, 1: 116, 1925.
3. CATEL, W.: Normale und pathologische Psychologie der Bewegungsvorgänge im gesamten Verdauungskanal. G. Thieme, Leipzig 1936.
4. CANESTRINI, S.: Über das Sinnesleben des Neugeborenen. Springer, Berlin 1913.
5. DARWIN, CH.: Biographische Skizze eines kleinen Kindes. Kleinere Schriften, 2. E. Krause, Leipzig 1886.
6. DENISOVA, M. P. and FIGURIN, N. L.: K voprosu o pervykh sočetael'nykh piščevykh refleksach u grudnykh detej (The first conditioned food reflexes in infants). *Vopr Genet Refleksol Pedolog*, 1: 81, 1929.
7. GAMPER, S. and UNTERSTEINER, F.: Über eine komplex gebaute postencephalische Hyperkinese und ihre möglichen Beziehungen zum oralen Einstellungsmechanismus. *Arch Psychiat*, 71: 282, 1924.
8. KASATKIN, N. I.: Rannije uslovnyje refleksy v ontogeneze u čeloveka (Early conditioned reflexes in human ontogenesis). Medgiz, Moscow, 1948.
9. KASATKIN, N. I., MIRZOJANC, N. S. and CHOCHITVA, A. P.: Ob orientirovočnykh uslovných refleksach u detej pervogo goda žizni (The conditioned orientation reflexes in infants during the first year of life). *Ž Vys Nerv Dejat*, 2: 192, 1953.
10. MARQUIS, D. P.: Can conditioned responses be established in the newborn infant? *Ped Sem & J Genet Psychol*, 39: 479, 1931.
11. MINKOWSKI, M.: Neurobiologische Studien an menschlichen Früchten. *Abderhaldens Hb Biol Arbeitsmeth V*, B, 511, 1928.
12. PAPOUŠEK, H.: Aktografie u malých kojenců (Activity recording in small infants). *Čs Pediatrie*, 11: 850, 1956.
13. — Metodika issledovanija piščevykh uslovných refleksov u detej v vozraste do šesti mesjacev (Method of studying conditioned food reflexes in infants during the first six months of their life). *Ž Vys Nerv Dejat*, 1: 143, 1959a.
14. — Podmíněné motorické potravové reflexy u kojenců (Conditioned food-seeking reflexes in infants). Dissertation. Charles University, Prague 1959b.
15. PEIPER, A.: Unbedingte und bedingte Reflexe der Nahrungsaufnahme. *Kinderärztl Praz*, 11: 507, 1958.
16. POPPER, S.: Studien über Saugphänomene. *Arch Psychiat Nervenhk*, 1: 231, 1921.
17. PRECHTL, H. F. R.: Die Kletterbewegungen

- beim Säugling. *Maschr Kinderheilk*, 101: 519, 1953.
18. PREYER, F.: Die Seele des Kindes. Th. Grieben, Leipzig 1895. 4th Ed.
19. RIPIN, R. and HETZER, H.: Frühestes Lernen des Säuglings in der Ernährungssituation. *Z Psychol*, 1-3: 82, 1930.
20. SCHEUDEL, F.: Wärme- und Kältereaktionen von neugeborenen Säuglingen. Dissertation. Greifswald, 1945.
21. SIKORSKI, I. A.: Die seelische Entwicklung des Kindes. Leipzig 1908.
22. STIRNIMANN, F.: Die Einstellreaktion beim Neugeborenen. *Jb Kinderheilk*, 5/6 326, 1937.
23. — Das Kind und seine früheste Umwelt. S. Karger, Basel 1947.

Submitted Dec. 8, 1960  
Accepted Jan. 26, 1961

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## Studies of the Immunological Relationship between the Antigens from Different Organs in Human Ulcerative Colitis<sup>1</sup>

by OVE BROBERGER

In a previous publication [1] it was reported that sera from children with ulcerative colitis usually contain antibodies which react with extracts from human colon, liver or kidney. The antibodies could be demonstrated by means of gel diffusion and haemagglutination techniques and behaved electrophoretically as gamma-globulins. The tissue extracts were prepared by extraction with phenol-water at 65°C and were supposed to contain lipopolysaccharides. This assumption was further substantiated by the fact that the extracted tissue components could be adsorbed onto sheep red cells without previous treatment of the cells with tannic acid [2].

Since the sera reacted with extracts from both colon, liver and kidney, it was desirable to learn whether the antigens from the different tissues were immunologically identical, related (cross-reacting), or different. Previous experiments, by means of gel diffusion techniques, had given certain indications that they could be identical or cross-reacting [1]. However, gel diffusion cannot be regarded as a sensitive tool for the study of antigenic identities in cases where knowledge of the optimal concen-

trations of antigen and antibody is not precise. This communication gives the results of a study of such relationships by means of haemagglutination inhibition.

### Methods

*Source and preparation of antigen.* Colon, liver and kidney were obtained within one hour after death from children (blood group O) who had died during their first day of life. The organs were extracted with phenol-water at 65°C as described elsewhere [1]. For coating of the red cells the lyophilized extracts were dissolved in buffered saline (phosphate buffer, pH 7.4) in a concentration of 0.5 mg/ml.

*Antiserum.* Serum from a child with ulcerative colitis was used as antiserum. It reacted with colon extract in a dilution of 1/64 and with liver extract in a dilution of 1/32. Before use, the serum was inactivated at 56°C for 20 minutes in order to destroy complement. Heterophile antibodies were removed by repeated absorption with washed sheep erythrocytes.

*Haemagglutination inhibition.* Sheep erythrocytes were coated with colon or liver extracts as described elsewhere [1]. The titrations were performed on perspex trays (MRC pattern). Serial dilutions of serum were made in buffered saline and aliquots of 0.1 ml of the dilutions were placed in the rows of holes on the tray. One-tenth of a millilitre aliquots of the antigenic extract were then added using a different concentration of antigen for each row of serum dilutions.

<sup>1</sup> This work has been supported by a grant from the Swedish Medical Research Council.

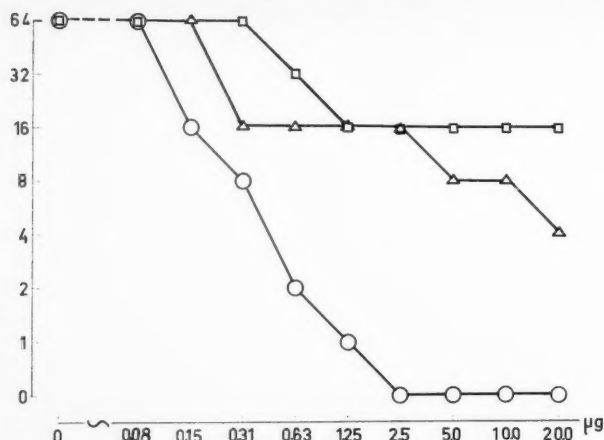


Fig. 1. Haemagglutination inhibition of sheep erythrocytes coated with colon extract after absorption of antiserum with colon, liver and kidney extract. *Abscissa*: Amount of antigenic extract ( $\mu\text{g}$ ) used for absorption of 0.1 ml of antiserum. *Ordinate*: Serum titre given as the reciprocal of highest serum dilution giving macroscopically visible haemagglutination. *Circles*: Haemagglutination of colon-coated cells after absorption of antiserum with colon extract. *Triangles*: After absorption with liver extract. *Squares*: After absorption with kidney extract.

Antigen dilutions were made in twofold steps from 1/1 (= 0.2 mg/ml) to 1/256. In controls, 0.1 ml of buffered saline instead of antigen was added to each of the serum dilutions. For absorption the mixtures were incubated at 37°C for 60 minutes. One-tenth of a millilitre aliquots of a 0.25% suspension of antigen-coated sheep erythrocytes (standardized in a Beckman spectrophotometer after haemolysis) were finally added to all samples. The trays were shaken and kept at 37°C for 60 minutes. After a second shaking the trays were left at 4°C overnight. The settling pattern of the red cells gave some indication of the degree of haemagglutination. However, the final judgement was always made on the appearance of the red cells after a gentle shaking. The serum titer is given as the reciprocal of the highest serum dilution giving macroscopically visible haemagglutination.

### Results

As can be seen from Fig. 1, unabsorbed serum caused haemagglutination of sheep erythrocytes coated with *colon* extract in

a serum titer of 64. When the serum was absorbed with 2.5  $\mu\text{g}$  of colon extract or more, the haemagglutination was completely inhibited. Using liver extract for absorption, complete inhibition could not be obtained under the present experimental conditions. The curve represented by squares reveals that the inhibitory capacity of the kidney extract was even less than that of the liver extract.

Fig. 2 shows the results of another experiment when the sheep red cells were previously coated with *liver* extract. In this case, the titer of the unabsorbed serum was 32. As is apparent from the shape of the curves, no significant difference between the inhibitory capacities of the colon and liver extract could be noted in an experiment of this type. Unfortunately, the small amount of kidney extract available prevented additional experiments with kidney-absorbed serum or with kidney-coated cells.



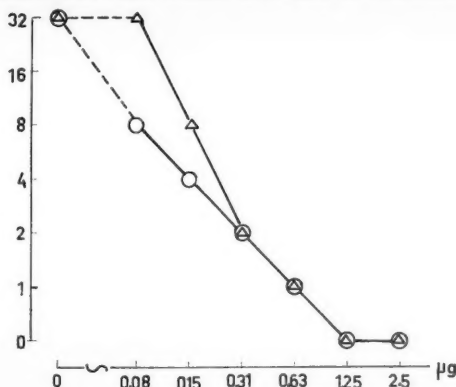


Fig. 2. Haemagglutination inhibition of sheep erythrocytes coated with liver extract after absorption of antiserum with colon and liver extract. *Abscissa*: Amount of antigenic extract ( $\mu\text{g}$ ) used for absorption of 0.1 ml of antiserum. *Ordinate*: Serum titre given as the reciprocal of highest serum dilution giving macroscopically visible haemagglutination. *Circles*: Haemagglutination of liver-coated cells after absorption of antiserum with colon extract. *Triangles*: After absorption with liver extract.

### Discussion

There are several explanations available for the results of the haemagglutination experiments depicted in Fig. 1. Thus one could assume that haemagglutination is brought about by the reaction of the antibodies with an immunologically identical antigen occurring in both colon, liver and kidney extracts. The greater inhibitory capacity, based on the amount of lyophilized powder used, of the colon extract as compared with that of the liver or kidney extracts would then be due to a larger amount of antigen in the colonic extracts. Secondly, the antigens occurring in the three organs could be different but immunologically related. In this case, haemagglutination inhibition would have been caused by the presence, in the liver and the kidney extracts, of antigens which cross react with the antibodies against the colon antigen. With other words, the results obtained in Fig. 1, with liver or kidney extracts, could be explained on the

basis of a lesser fit of these antigens with the anti-colon antibodies. However, a third possibility has also to be taken into consideration. In addition to antibodies against one or several common antigens present in colon, liver and kidney, the sera may also contain antibodies against antigen(s) present only in the colon. This would fit with the incomplete inhibitions obtained with the liver and kidney extracts in the experiments of Fig. 1. Moreover, this explanation is favoured by the results given in Fig. 2. The very similar inhibition curves obtained here with the colon and liver extracts seem to indicate that only a fraction of the antibodies which participate in the haemagglutination of *colon*-coated cells are involved in the haemagglutination of *liver*-coated cells. In any case, the complete inhibition of haemagglutination of *liver*-coated cells after absorption with colon extract also suggests that these two organs have at least one immunologically identical or similar antigen in common.

The occurrence of antibodies, in the sera of patients with ulcerative colitis, against several unrelated antigens would account for the fact, noted previously [1] that not all sera which react with colon extract also react with liver or kidney extract. However, the insufficient amount of antigen available has so far prevented an exploration of this problem. Any definite conclusions as to a possible multiplicity of the antigens and the antibodies have to await the results of further experiments.

### Summary

Sera from children with ulcerative colitis haemagglutinate sheep red blood cells

coated with phenol-water extracts of human colon, liver or kidney. It was found that the haemagglutination of colon-coated cells could be completely or partially inhibited by previous absorption of the sera with extracts of colon, liver or kidney. Likewise, the haemagglutination of liver-coated cells could be inhibited by means of extract from colon or liver. The results indicate that liver and colon contain at least one immunologically identical or similar antigen. In addition, certain preliminary results suggest that the sera contain antibodies against several distinct antigens, some of which are present only in the extracts of colon and not in those of liver or kidney.

### References

1. BROBERGER, O. and PERLMANN, P.: Auto-antibodies in human ulcerative colitis. *J Exp Med*, 110: 657, 1959.
2. RHODES, J. M., SORKIN, E. and BOYDEN, S.

V.: Studies on a haemosensitin from Mycobacterium Tuberculosis in zone electrophoresis. *Biochim Biophys Acta*, 2: 405, 1957. Personal communication.

Submitted April 11, 1961  
Accepted April 24, 1961

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## Concentration of Fibrinogen in the Plasma of Healthy and of Erythroblastotic and Hyperbilirubinemic Newborn Infants

by LARS ENGSTRÖM and LARS KAGER

Taylor (5) presented the data from the literature on the concentration of fibrinogen in the plasma of newborn infants. The mean values reported in the various studies range from 140 to 350 mg/100 ml, with individual observations from 50 to 800 mg/100 ml. The discrepancies are thus considerable. Taylor also made observations of the concentration of fibrinogen in the plasma during the first week of life of 45 healthy infants. He used the clot density technique of Losner *et al.* (2) and found a slight increase in mean concentration of fibrinogen during the first three days of life. (First day mean 231 and range 182-398, third day mean 264 and range 196-399.) Neither sex, birth weight, nor concentration of fibrinogen in the mother's plasma had any influence on the infant's initial concentration of fibrinogen. Taylor discussed the possibility of fibrinogen concentration being used as a measure of an infant's liver function.

Rice (4) reported an immature infant (birth weight 1900 g) with erythroblastosis and petechiae on the abdomen and face and with blood-tinged sputum. The clotting time was two hours. Addition of thrombin in excess resulted in the forma-

tion of a fine fibrinous network. After exchange transfusion of 25-35% of the blood, the plasma fibrinogen was 120 mg/100 ml. Rice concluded that the quantity of fibrinogen *before* the transfusion had either been appreciably lower, or that the blood had been initially free of fibrinogen.

Proceeding from Taylor's and Rice's results we have investigated whether there is any difference between the concentrations of fibrinogen in the plasma of healthy newborn infants and in infants with erythroblastosis or hyperbilirubinemia without blood-group incompatibility.

### Material and Methods

Samples for fibrinogen study were drawn from 39 infants by means of a polyethylene catheter introduced into the umbilical vein. Twenty of the subjects were healthy full-term infants; the samples from fourteen of these infants were collected within two hours after birth, while in the other six the study extended over the first three to six days of life.

Ten infants had erythroblastosis due to Rh or ABO immunization of the mother. In these cases the samples were collected within two hours of birth and *before* an exchange transfusion.

TABLE 1. *Fibrinogen concentration in plasma of erythroblastotic infants at birth (mg/100 ml).*

Degree of severity		No. of cases	Fibrinogen		
<i>Group 1</i>					
Coombs( + ); 0 exchange transfusion		1	270		
<i>Group 2</i>					
1 exchange transfusion		4	190, 230, 240, 310		
<i>Group 3</i>					
2 exchange transfusions		2	240, 300		
<i>Group 4</i>					
3 or more exchange transfusions		3	285, 290, 350		
<i>Groups 1-4</i>					
No. of cases	Range	Mean	SE	SD	
10	190-350	271	14.7	46.3	

Nine infants had exchange transfusions performed because of hyperbilirubinemia without blood-group incompatibility. Samples for fibrinogen determination were drawn before the first transfusion, which was between the third and sixth day of life.

The fibrinogen was determined by Morrison's syneresis method (3) as modified by Blombäck (1), a method not previously used in determination of fibrinogen in the plasma of newborn infants. The anticoagulant used in the collection of the samples consisted of 3.8% trisodium citrate (2 H<sub>2</sub>O) in a dilution of one part to nine parts of blood. To every ml of trisodium citrate 40 mg of lysin ethyl ester dihydrochloride were added, so that any fibrinolytic activity present in the sample was eliminated (1, 6).

In 33 infants hematocrit values were determined concurrently with collection of the sample for fibrinogen determination (centrifugation for 5 minutes at 10,000 r.p.m.).

## Results

### A. Healthy full-term infants examined within two hours of birth

Individual observations ranged from 175 to 285 mg/100 ml, giving a mean

value of 225. (Standard error  $\pm 10.0$ , standard deviation  $\pm 37.3$ , number of cases 14.) No difference was found between boys and girls.

One infant, who was excluded from the normal material because of mongolism, had the lowest fibrinogen concentration in plasma of any infant studied, i.e., 120 mg/100 ml.

### B. Healthy full-term infants examined during third to sixth day of life

The mean was 322, lowest 240 and highest 380 mg/100 ml. (Standard error 21.2, standard deviation 51.9, number of cases 6.)

### C. Erythroblastotic infants

These infants are classified in four groups in Table I according to the severity of the condition. Group 1 comprises the mildest cases whose Coombs test was directly positive but who did not require an exchange transfusion. The infants in Group 2 received one exchange transfu-

TABLE 2. *Fibrinogen concentration in plasma of infants with hyperbilirubinemia without blood-group incompatibility.*

Case no.	Non-conjugated bilirubin, mg/100 ml	Fibrinogen mg/100 ml
1	24.0	195
2	17.1	200
3	21.9	225
4	20.2	270
5	20.8	285
6	24.3	290
7	23.1	350
8	19.5	415
9	21.3	445

Case 1-9				
No. of cases	Range	Mean	SE	SD
9	195-445	297	30.0	89.8

sion, in Group 3 two, and in Group 4 at least three. The most seriously affected children are thus those in Groups 3 and 4. The table also shows the fibrinogen concentration at the time of the first transfusion. The individual values ranged from 190 to 350 mg/100 ml with mean 271. (Standard error 14.7, standard deviation 46.3, number of cases 10.)

#### D. *Hyperbilirubinemic infants without blood-group incompatibility*

The severity in terms of the non-conjugated serum bilirubin concentration at the time of the first exchange transfusion, which immediately followed the drawing of the sample for fibrinogen study, is shown in Table 2. The fibrinogen concentrations ranged from 195 to 445 mg/100 ml, mean 297. (Standard error 30.0, standard deviation 89.8, number of cases 9.)

In 33 cases the plasma fibrinogen concentration was correlated to the hematocrit value at the time of drawing the sample, without regard to the above classification (Table 3).

## Discussion

The results show that the concentration of fibrinogen in plasma was not lower in erythroblastotic than in healthy full-term infants. Indeed, the most severely affected infants proved to have fairly high fibrinogen levels.

Statistical analysis revealed that there was no correlation between hematocrit value and fibrinogen concentration as shown in Table 3. Thus a low hematocrit value in conjunction with erythroblastosis did not affect the fibrinogen concentration.

Samples for fibrinogen determination were drawn within two hours of birth. No account has been taken in this study of the possibility that the fibrinogenolytic activity in erythroblastic infants might become elevated after birth. Hemorrhagic tendencies were not observed clinically in any case.

Proceeding from Taylor's postulate that the plasma fibrinogen may be an index of the liver function at birth, we determined the concentration of fibrinogen in

TABLE 3. *Relationship between haematocrit value and plasma fibrinogen concentration*

Case no.	Hematocrit, %	Fibrinogen, mg/100 ml	Case no.	Hematocrit, %	Fibrinogen, mg/100 ml
1	32	300	18	55	195
2	35	350	19	56	285
3	35	310	20	56	340
4	35	290	21	56	325
5	43	285	22	57	290
6	43	380	23	57	270
7	46	240	24	57	270
8	46	445	25	58	190
9	46	290	26	59	290
10	47	240	27	59	415
11	49	210	28	59	250
12	50	325	29	60	175
13	50	350	30	60	370
14	52	230	31	61	180
15	54	215	32	62	275
16	55	230	33	70	120
17	55	240			

the plasma of a group of infants with neonatal hyperbilirubinemia without blood-group incompatibility. The indication for exchange transfusion was here an elevated, non-conjugated bilirubin level in serum (Table 2), i.e. an expression of inadequate hepatic capacity. Since the transfusions and determinations of fibrinogen concentration were not done in these infants until the third to sixth day of life, the fibrinogen concentration was also studied in a group of infants of the same age but without icterus. No change was observed in plasma fibrinogen as a result of elevated, non-conjugated serum bilirubin. Nor was there any correlation between fibrinogen concentration and the quantity of non-conjugated bilirubin.

Apart from the fibrinogen value of the mongoloid infant, all fibrinogen levels fell within the limits found by Taylor for healthy children during the first week of life.

Our results, therefore, do not support

Rice's hypothesis that Rh-immunization is accompanied by a low concentration of fibrinogen in the plasma of the affected infant—nor that the fibrinogen concentration changes as a result of hyperbilirubinemia not caused by blood-group incompatibility. This does not exclude the possibility, of course, that severe cases of erythroblastosis or hyperbilirubinemia may be complicated by afibrinogenemia due, for example, to intravascular clotting or fibrinogenolysis without direct association with the basic disease. In erythroblastosis and hyperbilirubinemia in general, however, the fibrinogen concentration in plasma is not a test of functional value, though this does not preclude the necessity of complete investigation of coagulation, including determination of fibrinogen concentration in the plasma, in all cases of clinically observed hemorrhagic diathesis in infants. But, as already stated, we had no case of hemorrhagic diathesis in our material.

## Summary

The concentration of fibrinogen in the plasma of 39 infants during the neonatal period was determined by Morrison's syneresis method as modified by Blombäck. The samples were drawn from the umbilical vein by catheterization. The results, expressed in mean and standard error of the mean, were as follows:

- A. Healthy full-term infants at birth:  $225 \pm 10.0$  mg/100 ml.
- B. Healthy full-term infants during

third to sixth day of life:  $322 \pm 21.2$  mg/100 ml.

- C. Erythroblastotic infants at birth:  $271 \pm 14.7$  mg/100 ml.
- D. Hyperbilirubinemic infants without blood-group incompatibility during third to sixth day of life:  $297 \pm 30.0$  mg/100 ml.

No statistically significant difference was found between healthy and erythroblastotic or hyperbilirubinemic infants as regards concentration of fibrinogen in the plasma.

## References

1. BLOMBÄCK, B.: On the properties of fibrinogen and fibrin. *Ark Kemi*, 12: 99, 1958.
2. LÖNNER, S., VOLK, B. V., JACOBI, M., and NEWHOUSE, S.: Spectrophotometric studies on clot density. *J Lab Clin Med*, 38: 28, 1951.
3. MORRISON, P. R.: Preparation and properties of serum and plasma proteins. IV. Some factors influencing the quantitative determination of fibrinogen. *J Amer Chem Soc.*, 69: 2723, 1947.
4. RICE, W. G.: Deficient prothrombin and fibrinogen in fatal erythroblastosis fetalis. *J Pediatr*, 42: 231, 1953.
5. TAYLOR, P. M.: Concentration of fibrinogen in the plasma in normal newborn infants. *Pediatrics*, 19: 233, 1957.
6. TROLL, W., SHERRY, S., and WACHMAN, J.: The action of plasmin on synthetic substrates. *J Biol Chem*, 208: 85, 1954.

Submitted Jan. 2, 1961

Accepted Jan. 26, 1961

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## Arteriographic Studies in Children with Cerebral Palsy

by S. BRANDT, S. BRÜNNER and V. WESTERGAARD-NIELSEN

Cerebral angiography is being used to an increasing extent in neurologic disease because it affords such an excellent supplement to other diagnostic methods. Until the percutaneous method of injecting the contrast material was described about 15 years ago, there was marked reluctance to subject children to angiography. For this reason pneumoencephalography has been preferred where information on cerebral pathology was wanted in children. Recently two papers on encephalographic findings in spastic children have been published (3, 9). On the other hand, angiographic studies on children with cerebral palsy or studies correlating angiographic with encephalographic findings have not been reported although some recent authors have reported in general on carotid angiography in children (6, 7, 10, 11). We have, therefore, collected a series of 24 patients from the Cerebral Palsy Clinic at the Orthopaedic Hospital in Copenhagen, in whom arteriographic and encephalographic studies had been performed in some of the radiological and neurosurgical departments in Copenhagen and at the Radiological Department of the Copenhagen County Hospital in Gentofte, usually prior to the time of examination at the Cerebral Palsy Clinic.

Because of the widely different pathology it was anticipated that the angiograms would not show specific changes; indeed the findings were protean. Nevertheless we felt that it might be of interest to compare the angiograms with encephalograms obtained at approximately the same time.

### Material

The angiograms were mainly obtained from children with cerebral palsy of postnatal origin because patients of this category are more likely to be examined in neurosurgery departments where angiography is a rather routine diagnostic procedure. In a series of 628 children with cerebral palsy examined at the Cerebral Palsy Clinics at the Orthopaedic Hospitals in Copenhagen and Aarhus, Brandt & Westergaard-Nielsen (1) found 13% postnatal cases, whereas in the present series 14 of 24 children i.e. a little over one-half, were postnatal. Six were congenital (i.e. presumably prenatal), and only four perinatal in onset. The distribution of patients on the basis of the nature and extent of the disturbance of coordination is shown in Table 1. The series comprises 12 girls and 12 boys who ranged in age from 6 months to 15 years at the time of the angiographic examination. Only four were under 2 years of age.

TABLE 1. *Symptomatic classification.*

<i>Spastics</i> (= pyramidal)	19
Left-sided	9
Right-sided	5
Predominantly left-sided	4
Predominantly right-sided	0
No difference between right and left	1
<i>Athetotics</i> (= extrapyramidal)	2
<i>Mixed type</i>	3
Total	24

### Technique

The 24 patients underwent a total of 40 carotid angiographies, 29 right-sided and 11 left-sided. All the examinations were performed by the percutaneous technique under general anaesthesia.

Encephalography had been performed by the suboccipital or lumbar route or as ventriculography a total of 39 times. Two patients did not have encephalography. As is apparent from the figures, these procedures were repeated in a majority of the patients. Eight had two angiographies, five had three, and two had four angiographic examinations.

### Results

#### (a) *Patients with ventricular dilatation*

In 18 out of the 24 children, encephalography revealed enlargement of the ventricular system. It was symmetrical in seven, whereas in seven there was right-sided, and in four left-sided dilatation only. These 18 children had a total of 25 right-sided and five left-sided angiographic examinations. The uneven distribution of right- and left-sided procedures is in accordance with the fact that in children with cerebral palsy there is a preponderance of postnatal, left-sided, spastic hemiplegia (1). The arteriographic findings in these patients are summarized in Table 2.

TABLE 2. *Arteriographic findings in patients with ventricular dilatation.*

Elevation, distension, and possibly shift of the anterior cerebral artery	8
Vascular malformation	2
Thrombosis in the anterior cerebral artery	1
Agenesis of the corpus callosum	2
Normal	5

From the table it may be seen that of 18 patients with ventricular dilatation, pathologic arteriographic findings were found in 13, a relatively high proportion, possibly because of the somewhat stricter indications for arteriography in children.

In one of the cases in the first group (elevation, distension, and possibly shift of the anterior cerebral artery), it had been suggested in the report of the arteriograms—supported by X-ray skeletal changes—that there might be an expansive lesion. This was later proved. Another case in the same group came to autopsy 15 months after the last radiological examination; an angioma racemosum arterio-venosum was found in the right temporal lobe and a haematoma in the corpus striatum on the right with bleeding into the right lateral ventricle. Furthermore, there was hydrocephalus (the case has been reported by Christensen & Brandt (2)). Even with the knowledge of the post mortem findings, the lesion could not be demonstrated in a review of the right-sided angiograms; however, the afferent vessels, especially the middle cerebral artery, were rather prominent for a 1-year-old baby, and the efferent vessels were extremely well developed. This finding demonstrates how difficult it may be to visualize angioma racemosum on the angiograms obtained

by the usual three-film technique owing to the great speed at which the blood runs through the numerous arterio-venous shunts (5, 8). The chances of "catching" an angioma racemosum are considerably greater when using rapid serial angiography (4).

(b) *Patients without ventricular dilatation*

Four out of the 24 children showed no signs of ventricular enlargement or other encephalographic abnormalities. In three of these cases the angiograms were normal, while one showed periarterial flow of contrast with non-filling of the anterior cerebral artery.

(c) *Non-encephalographed patients*

As already mentioned two children did not have encephalography. These two angiographies also showed the combination of periarterial flow of contrast with non-filling of the anterior cerebral artery.

(d) *Patients with clinical hemiplegia*

All the children with *left-sided* spastic hemiplegia belonged to the group with bilateral or right-sided dilatation of the ventricular system. In addition, one child of this category did not have encephalography. The arteriographic findings in this group of left-sided spastic hemiplegia are summarized in Table 3.

Two characteristic cases illustrate the findings in this group:

The first patient was a boy, aged 21 months, who had been delivered by caesarean section. From the age of 3 months he began to have clonic twitching in the left arm, and spastic left-sided hemiplegia was noted. His general development was retarded. At

TABLE 3. *Arteriographic findings in patients with clinical, left-sided hemiplegia.*

Distension and elevation of the right-sided anterior cerebral artery	1
Aneurysm of the right internal carotid	1
Shift of the right anterior cerebral artery to the right	2
Thrombosis in the right internal carotid	2
Normal	3

the age of 4 months, he had subdural puncture, evacuating a hygroma. Ventriculography (Fig. 1) showed ample surface air on the left, no surface air on the right, a slight shift of the ventricular system to the right, and dilatation mainly on the right. Angiography at the age of 21 months revealed displacement of the anterior cerebral artery to the right. Both anterior cerebral arteries filled. By compression, filling was also obtained of the left middle cerebral artery. The peripheral vessels in the area supplied by the right medial cerebral artery did not extend quite to the tabula interna. In view of the displacement to the right of the anterior cerebral artery these findings suggest cortical atrophy and rather exclude a recurrence of the hygroma.

An 11-year-old boy had been in good health until the age of 9, when he sustained two cranial injuries in rapid succession. He then developed left-sided spastic hemiparesis. Right-sided carotid angiography revealed a saccular aneurysm at the bifurcation of the internal carotid artery. Craniotomy was performed, excising the aneurysm and clamping the anterior cerebral artery with clips (Fig. 2).

TABLE 4. *Arteriographic findings in patients with clinical, right-sided hemiplegia.*

Suspicion of astrocytoma in the left temporal lobe	1
Shift of the anterior cerebral artery to the contralateral side	1
Normal	3

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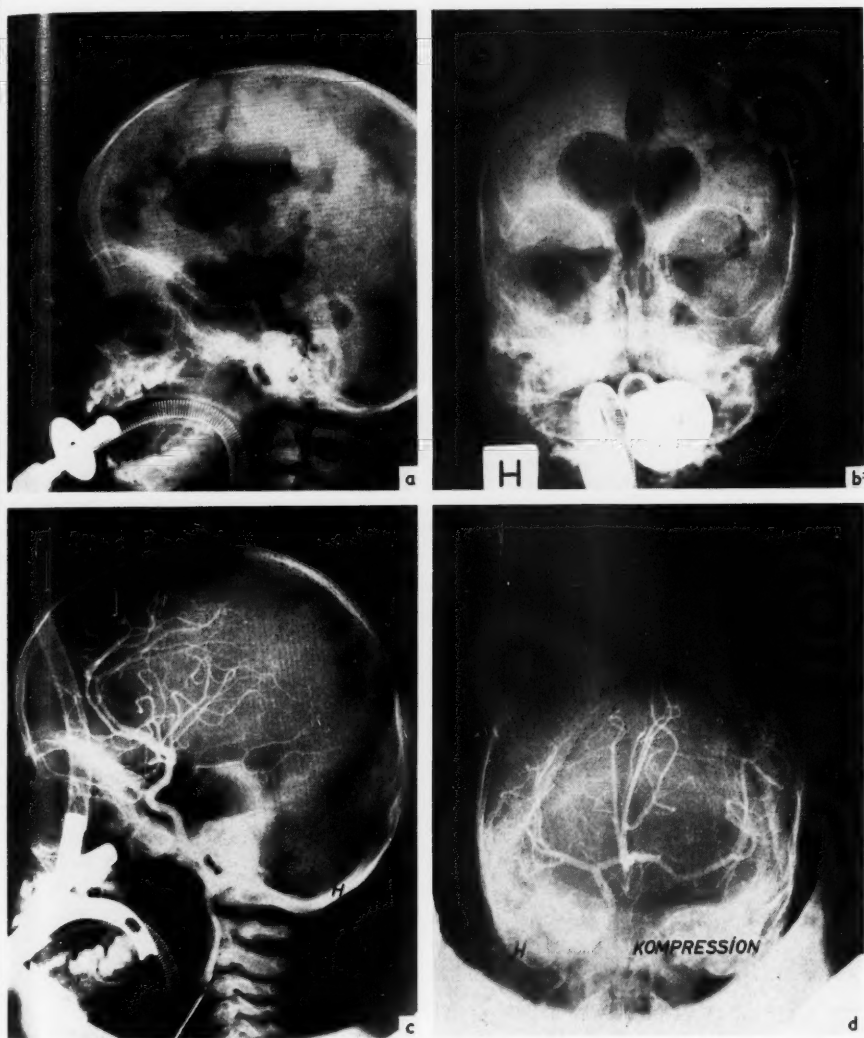


Fig. 3. (a) Lateral ventriculogram and (b) antero-posterior ventriculogram of a 21-month-old boy with a left-sided hemiplegia, showing a dilated ventricular system, especially on the right side. There is a small displacement towards the right and abundant surface air over the left hemisphere—none over the right. (c) and (d) Right carotid arteriogram showing a displacement of the anterior cerebral artery towards the right. The middle cerebral vessels do not reach the inner surface of the skull on the right side. Both anterior cerebral arteries are filled, distended and elevated, indicating cortical atrophy.



Fig. 2. (a) and (b) Right carotid arteriography on an 11-year-old boy with a left-sided hemiplegia, shows a sacculate aneurysm at the internal carotid artery's site of division. (c) Shows the same case immediately following operation with attached Cushing's clips. There is no filling of anterior cerebral artery.

The relation between *right-sided* spastic hemiplegia and the arteriographic findings may be seen in Table 4. All three children with normal arteriographic findings also had normal encephalograms. The following

report describes the findings in one of the two patients with an abnormal angiogram.

A 12-year-old boy had severe clinical signs consisting of right-sided spastic paresis with hyperkinesia of the limbs from the first

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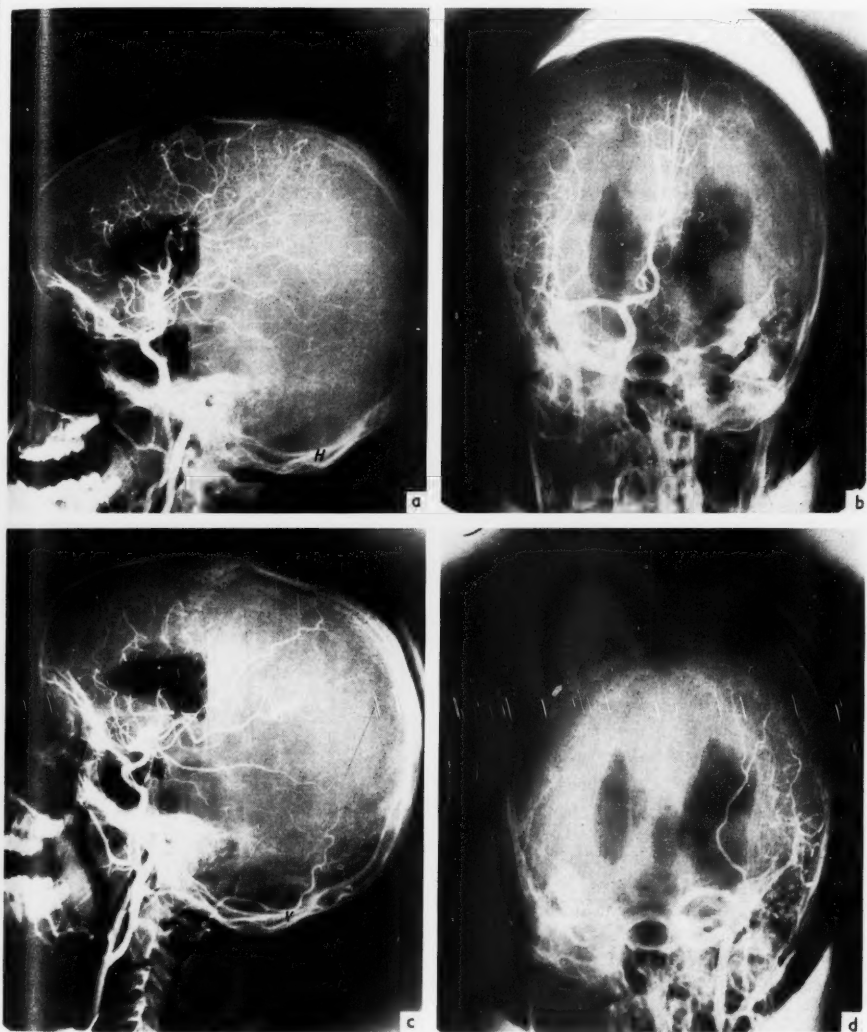


Fig. 3. (a) and (b) Right carotid arteriography on a 12-year-old boy with a right-sided spastic hemiplegia, performed one day after a lumbar encephalography, shows the anterior cerebral artery well developed and passing steeply up between the lateral ventricles supplying both hemispheres. The preceding encephalography had shown dilatation of both lateral ventricles, primarily of the right, together with agenesis of the corpus callosum. (c) and (d) Left carotid arteriography shows no filling of the left anterior cerebral artery.



year of life. From the age of 9, focal seizures at times with loss of consciousness. Lumbar encephalography showed enlargement of the lateral ventricles, more marked on the left. The third ventricle showed changes typical of agenesis of the corpus callosum. Bilateral carotid arteriography disclosed a steep course of the anterior cerebral artery on the right. On the lateral view it was superimposed upon the lateral ventricles. On the antero-posterior views there was a compensatory development of the right anterior cerebral artery, as the left one has probably never developed (Fig. 3).

#### (e) *Patients with spastic tetraplegia*

Another characteristic group among spastic children is tetraplegia. The present series included three children with spastic tetraplegia, one with spastic tetraplegia combined with choreo-ataxia and one combined with athetosis.

A 5-year-old boy was found to have epidural haematoma extending over a large part of the right hemisphere. He came to operation without preceding arteriography, since there was a vital indication for immediate neurosurgery. His acute condition improved immediately after the operation, but spastic tetraplegia persisted, and the boy showed slight mental impairment. After a repeated, less severe cranial injury carotid angiography was performed 3 months after the operation. This was normal, indicating that the tetraplegia could not be due to a recurrence of a localized haematoma.

A 9-year-old boy had previously shown normal development. He was admitted to a department of neurosurgery after having been run over by a car, in a state of profound unconsciousness, with brain-stem seizures and spastic tetraplegia. He recovered only slowly, and after bilateral arteriography, supplemented by lumbar encephalography, he was transferred to a department of general surgery because of a complicating fracture of the leg. The radiological investi-

gation had shown only slight enlargement of the ventricular system and no apparent extracerebral haematoma. From the surgical department the patient was transferred to a training department, but as he exhibited increasing lack of concentration, impairment of memory, and unrest, he was referred to a paediatric department and right-sided carotid arteriography was repeated 8 months after the injury. The films now showed a subdural haematoma over the right hemisphere with additional suspicion of intracerebral haemorrhage (Fig. 4). Although not all the bilateral clinical manifestations could be accounted for by this haemorrhage, the repeated arteriography had at least detected a secondary pathology which could now be removed.

The arteriograms have given only a partial explanation of the tetraplegic symptom complex, but in one case they demonstrated and localized alarming haemorrhage and in another case afforded guidance for neurosurgery.

#### Summary and Conclusion

Our study of arteriograms and pneumoencephalograms from 24 children with cerebral palsy leads us to conclude that arteriography—however useful it may be in the acute and subacute stage of the disorder—offers very little more information than pneumoencephalography in the stationary stage of the disease. Ventricular dilatation may be suspected from the course of the arterial trunks and a shift may indicate atrophy of the involved hemisphere. Occasionally, however, indication of a vascular abnormality may be found, which would be undiagnosed by pneumoencephalography.

From some of our observations we are tempted to recommend that arteriography should be preferred to pneumoencephalography in patients with cerebral palsy in

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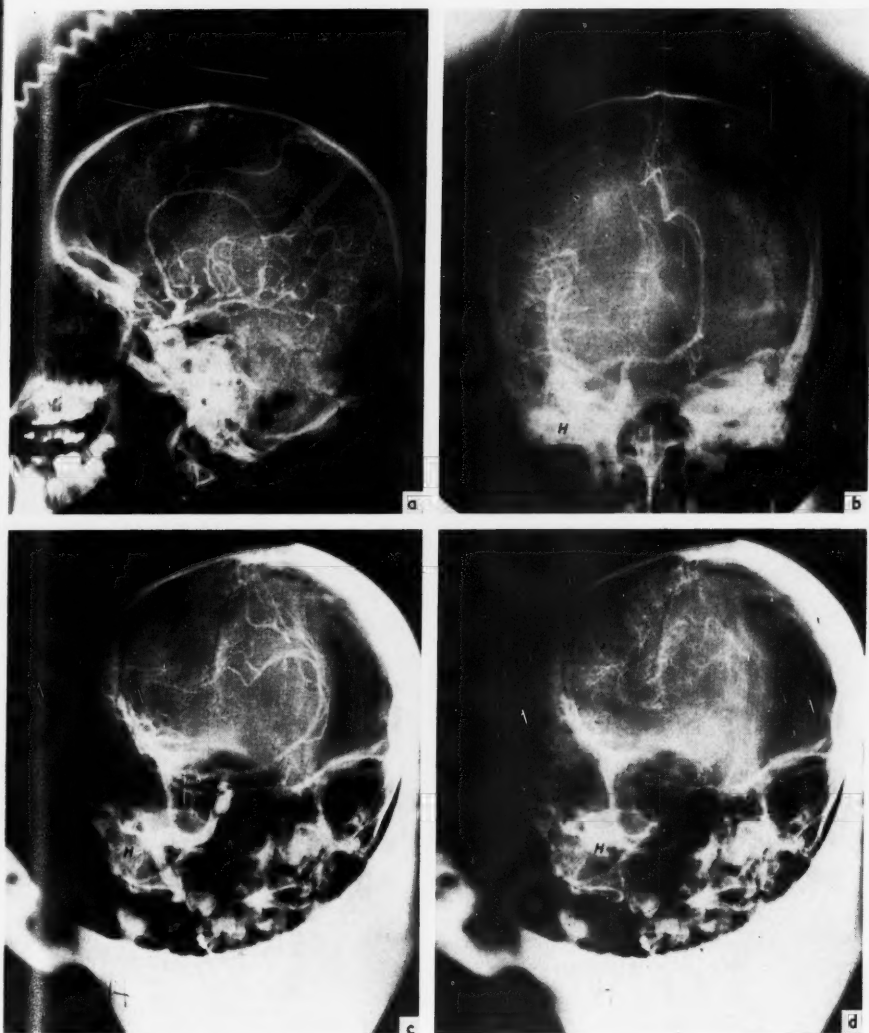


Fig. 4. Right carotid arteriography on a 9-year-old boy with spastic tetraplegia, which he developed 8 months after a skull trauma, shows a large subdural haematoma over the right hemisphere together with a pronounced displacement of the right anterior cerebral artery towards the left.

(1) cases of hemiplegia, especially with recurrent acute symptoms and exacerbations, (2) cases complicated by focal epileptic manifestations and (3) in persons in whom the symptoms of cerebral palsy

may be of rather recent date, perhaps not yet completely restored from their acute disease and in whom suspicion of overlooked pathology or complicating bleeding exists.

## References

1. BRANDT, S. and WESTERGAARD-NIELSEN, V.: Etiological factors in cerebral palsy and their correlation with various clinical entities. *Danish Med Bull*, 5: 47, 1958.
2. CHRISTENSEN, E. and BRANDT, S.: Recidiverende acut infantil hemiplegi forårsaget af dybt hemisphereangiom. *Nord Med*, 62: 1574, 1959.
3. DENHOFF, E., HOLDEN, R. H. and SILVER, M. L.: Prognostic studies in children with cerebral palsy. *JAMA*, 161: 781, 1956.
4. GREITZ, T.: A radiologic study of the brain circulation by rapid serial angiography of the carotid artery. *Acta Radiol (Stockh)*, Suppl. 140: 70, 1956.
5. HAMBY, W. B.: Rapport et discussions du premier congrès international des sciences neurologiques. Bruxelles 193, 1957.
6. PARATZ, E. and SZENASY, J.: Über die Carotis-angiographie in Kindesalter. *Acta Neurochir (Wien)*, 7: 350, 1959.
7. POSER, CH. M. and TAVERAS, J. M.: Clinical aspects of cerebral angiography in children. *Pediatrics*, 16: 73, 1955.
8. ROSNER, S.: The pathology and surgical treatment of complicated cerebral palsy. *Arch Pediat*, 75: 1, 1958.
9. SALOMONSEN, L., ECK, S. and SKATVEDT, M.: Pneumoencephalographic findings in cerebral palsy, epilepsy and oligophrenia. *Ann Paediat Fenn*, 3: 602, 1957.
10. SCHIEFER, W. and VETTER, K.: Das zerebrale Angiogram in den verschiedenen Altersstufen. *Zbl Neurochir*, 17: 231, 1957.
11. TAVERAS, J. M. and POSER, CH. M.: Roentgenologic aspects of cerebral angiography in children. *Amer J Roentgenol*, 82: 371, 1959.

Submitted January 9, 1961  
Accepted February 16, 1961

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## Respiratory Studies in Children

## III. Variability of the Lung Volumes in Healthy Children

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In a previous paper (3), the variability of the functional residual capacity ( $V_{FRC}$ ), one of the components of the lung volume, has been described and discussed.

The purposes of this work are to define the variability of the values of the other lung volumes in healthy children and to compare the variability with similar volumes in adults, since in many pulmonary diseases determination of the lung volumes is used not only for the diagnosis but also

for longitudinal survey and to estimate efficiency of therapeutic procedures (6). It therefore seems useful to know exactly the limits of the day-to-day physiological variation before drawing conclusions.

## Definitions and Nomenclature

The nomenclature is that proposed by a committee of respiratory clinical physiologists headed by Pappenheimer (5) (Fig. 1).

1. The vital capacity ( $V_{VC}$ ) was measured

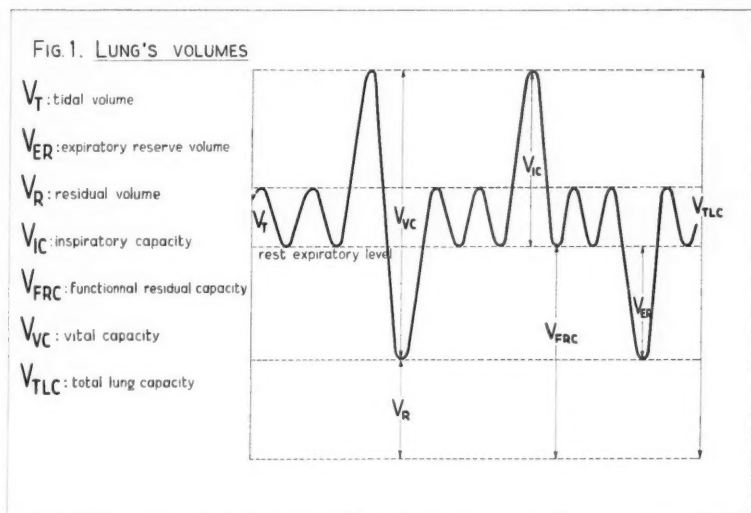


Fig. 1.

TABLE 1. Lung volumes in healthy adults. Time interval between the first (I) and the second (II) assay: 4 days; between the second and the third (III): 6 days. See Fig. 1 for symbols. All the volumes are ml BTPS.

Subject no.	Standing height, cm	I	II $V_{FRC}$	III	I	II $V_{IC}$	III
1	182	2387	3173	2831	5214	5005	5029
2	162	1840	2056	2159	3318	3160	3199
3	183	2884	3157	3110	4630	4390	3815
4	165	2120	1769	1714	3081	3081	2923
5	159	1659	1547	1970	3121	3279	3239
6	158	1931	1714	1900	2607	2765	2647
7	174	3150	2925	2779	3800	3753	4069
8	158	2318	2373	2376	2489	2528	2686
9	160	1762	1444	1702	2607	2489	2528
10	176	3624	3483	3069	3950	4108	3950
		$V_{VC}$			$V_{ER}$		
1		6360	6201	6412	1146	1196	1383
2		4268	4110	4304	950	950	1105
3		6129	6010	5355	1499	1620	1540
4		4187	3990	3832	1106	909	909
5		3832	3793	3990	711	514	751
6		3516	3555	3694	909	790	1047
7		5400	5412	5531	1600	1659	1462
8		3832	3832	3832	1343	1304	1146
9		3437	3279	3397	830	790	869
10		6083	6281	6123	2133	2173	2173
		$V_R$			$V_{TLC}$		
1		1241	1977	1448	7601	7178	8069
2		890	1106	1054	5158	5216	5358
3		1385	1537	1570	7514	7547	6923
4		1014	860	805	5201	4850	4637
5		948	1033	1219	4780	4826	5209
6		1022	924	853	4538	4479	4547
7		1550	1266	1317	6950	6678	6848
8		975	1069	1230	4807	4901	5062
9		932	654	833	4369	3933	4230
10		1491	1310	1196	6574	7591	6019

as the sum of the greatest inspiratory capacity ( $V_{IC}$ ) and the greatest expiratory reserve volume ( $V_{ER}$ ), chosen from three attempts at maximum inspiration followed by a maximum expiration.

In a previous paper (4), 64 records of vital capacity collected in 28 healthy and diseased children were critically analysed. The conclusions were that it is practically impossible to systematically obtain the  $V_{VC}$  beginning with a deep expiration, and furthermore it is

equally difficult to get three similar consecutive records. The latter is a requirement of many authors who have studied adults (1, 5). As a result of this analysis,  $V_{VC}$  in children is calculated by adding the largest  $V_{IC}$  and the largest  $V_{ER}$ , chosen anywhere on the spirogram.

2. The functional residual capacity ( $V_{FRC}$ ) represents the volume of air which is left in the lung after a normal expiration during quiet breathing.

TABLE 2. Lung volumes in healthy girls. Four-hour interval between the two italicized results. Between these and the third: four-day interval.

III	Subject no.	Standing height, cm	I	II V <sub>FRC</sub>	III	I	II V <sub>IC</sub>	III
	1	147	1249	1123	1320	2014	2053	2170
5029	2	150	1115	1031	1252	1817	2113	1915
3199	3	155	1788	1881	1642	2133	2054	2090
3815	4	104	650	630	674	672	632	849
2923	5	145	1609	1446	1665	2291	2409	1935
3239	6	164	1747	1900	2015	2547	2706	2960
2647	7	153	2093	2098	2417	2271	1738	1817
4069	8	155	1156	1320	1287	2193	2024	2212
2686	9	160	2323	2167	2180	2804	3160	2955
2528	10	158	1389	1249	1327	2375	2212	2820
3950	11	129	714	770	736	1280	1340	1323
				V <sub>VC</sub>			V <sub>ER</sub>	
1383	1		2646	2607	2585	632	554	415
1105	2		2686	2666	2410	869	553	495
1540	3		3239	2844	2880	1105	790	790
909	4		864	968	1007	192	336	158
751	5		3338	3456	3195	1047	1047	1260
1047	6		3990	3674	3712	1443	968	752
1462	7		3377	2844	3121	1106	1106	1304
1146	8		3042	3002	3002	849	978	790
869	9		3910	4226	4097	1106	1066	1142
2173	10		3436	3041	3374	1061	829	554
	11		1712	1700	1738	440	360	415
				V <sub>R</sub>			V <sub>TLC</sub>	
8060	1		617	569	905	3263	3176	3490
5358	2		246	478	757	2932	3144	3167
6925	3		683	1091	852	3922	3935	3732
4637	4		458	294	516	1322	1262	1523
5209	5		562	399	405	3900	3855	3600
4547	6		304	932	1263	4294	4606	4975
6848	7		987	992	1113	4364	4836	4234
5062	8		307	342	497	3349	3344	3499
4230	9		1217	1101	1038	5127	5327	5135
6019	10		328	420	773	3764	3461	4147
	11		274	410	321	1994	2110	2059

3. The residual volume ( $V_R$ ) is calculated as the difference between  $V_{FRC}$  and  $V_{ER}$ .

4. The total lung capacity ( $V_{TLC}$ ) is the sum of  $V_R$  and  $V_{VC}$ .

### Methods

1. With the nose closed by a nose-clip, the subject breathes through a mouthpiece into a spiographic system, without valves, but

with a fan-type pump. The system is similar to that of Engström *et al.* (2); the differences between the two have been described in a previous paper (3).

2. A closed circuit with helium as the test gas was used for the determination of the functional residual capacity ( $V_{FRC}$ ) (3).

3. All the values were corrected to body temperature, ambient barometric pressure, saturated with water vapor (BTPS).

TABLE 3. Lung volumes in healthy boys. Four-day interval between the first (I) and the second (II) assay. Six-day interval between the second and the third (III).

Subject no.	Standing height, cm	I	II $V_{FRC}$	III	I	II $V_{IC}$	III
1	150	1549	1650	1545	2173	2445	2232
2	125	861	863	874	1560	1620	1541
3	140	1223	1626	1210	1580	1343	1486
4	143	1177	1155	1118	1716	1857	2013
5	149	1556	1482	1537	2205	2105	1757
6	171	2470	2430	2494	3279	3418	3529
7	159	2310	2432	2380	2054	2250	2054
8	131	1137	1052	1016	1224	1305	1380
9	142	1248	1259	1251	2050	1890	2014
10	140	930	884	926	1797	1956	1896
11	137	1163	1258	1086	2032	1953	2251
12	136	1049	1040	1038	1797	1910	2073
		$V_{VC}$			$V_{ER}$		
1		3156	3195	3141	983	750	809
2		1916	1955	1976	356	335	433
3		2252	2094	1955	672	751	475
4		2726	2607	2469	1010	750	454
5		3035	2937	2666	830	832	909
6		4622	4700	4995	1343	1282	1473
7		3360	3080	3042	1306	830	988
8		1935	1817	1695	711	512	315
9		2622	2404	2606	572	514	592
10		2469	2489	2331	672	533	435
11		3002	2804	3121	970	851	870
12		2627	2464	2528	830	554	435
		$V_R$			$V_{TLC}$		
1		566	900	736	3722	4095	3877
2		505	528	439	2421	2483	2415
3		551	375	735	2803	2469	2699
4		167	405	664	2893	3012	3133
5		726	650	628	3761	3587	3294
6		1127	1148	1019	5749	5848	6014
7		1004	602	1392	4364	3682	4434
8		426	540	701	2361	2357	2396
9		676	745	659	3298	3149	3265
10		258	351	491	2727	2840	2822
11		193	407	216	3195	3211	3337
12		219	486	583	2846	2950	3111

### Material

1. Sixty-nine determinations were obtained while in the sitting position on 23 apparently healthy children, ranging in age from 6 to 15 years. Three assays were made on each patient with an interval of some hours

to several days. The  $V_{FRC}$  of each assay equals the mean of duplicate determinations.

2. Thirty determinations were obtained on 10 apparently healthy young adults (students, nurses, etc.) with the same procedure as on children for comparison of the "physiological variation".

TABLE 4. Mean values of the lung volumes in healthy adults, boys and girls. For details: see Tables 1 to 3. The values are: mean value ( $M$ ), standard error ( $E$ ) and standard deviation ( $S.D.$ ).

III		Adults (10 cases)			Boys (12 cases)			Girls (11 cases)			
		M.	E.	S.D.	M.	E.	S.D.	M.	E.	S.D.	
2232	V <sub>VC</sub>	I	4704	± 368	1162	2810	± 211	730	2931	± 281	932
1541		II	4646	± 376	1188	2712	± 219	760	2820	± 269	891
1480		III	4647	± 305	966	2710	± 247	857	2829	± 264	867
2015	V <sub>IC</sub>	I	3482	± 288	911	1956	± 146	507	2056	± 181	600
1757		II	3456	± 265	838	2004	± 158	547	2040	± 201	666
3520		III	3408	± 252	796	2018	± 160	553	2095	± 198	657
2054	V <sub>ER</sub>	I	1223	± 136	431	855	± 83	288	895	± 107	355
1380		II	1190	± 143	453	708	± 70	244	780	± 86	285
2014		III	1238	± 132	418	684	± 98	335	734	± 113	374
1896	V <sub>R</sub>	I	1145	± 79	250	544	± 100	317	535	± 90	312
2251		II	1174	± 119	376	639	± 97	320	595	± 69	238
2073		III	1123	± 87	275	767	± 92	305	687	± 85	294
809	V <sub>TLC</sub>	I	5749	± 402	1270	3345	± 277	959	3476	± 328	1088
435		II	5719	± 436	1378	3307	± 276	956	3551	± 458	1519
475		III	5680	± 393	1241	3399	± 290	1006	3604	± 328	1089
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## Results

The values for  $V_{FRC}$  have been presented and discussed in a previous paper (3). The error of the method (standard deviation of the differences between duplicate determinations, divided by the square root of two) expressed in per cent of the corresponding  $V_{FRC}$  is 2.5 % for adults and boys and 3.0 % for girls.

There was no statistical difference in the mean values of  $V_{FRC}$  in each of the three assays for each subject. Since no differences in mean values were found, the physiological variation<sup>1</sup> may be calculated.

Taking only the assays performed at four-day intervals, the physiological variation is 180 ml for the adults, 52 ml for the boys and 84 ml for the girls.

When expressed in per cent of the mean

<sup>1</sup> Standard deviation of the individual differences:  $\sigma_d = \sqrt{S(x - \bar{x})^2 / (n - 1)}$ . Physiological variation:  $\sigma_d / \sqrt{2}$ .

of the first  $V_{FRC}$  in each group this variation is 7.5, 3.7 and 5.8 %, respectively.

The values for  $V_{VC}$  are given in Table 4. The individual differences ( $V_{VC}I - V_{VC}II$ ) and ( $V_{VC}II - V_{VC}III$ ) and mean differences were calculated. The first minus the second  $V_{VC}$  for both boys and girls at four-day intervals is probably significant. No other statistical differences were found.

The values of physiological variation, excluding the two groups where statistical differences in means exist, are shown in Table 5.

TABLE 5. Physiological variations of the vital capacity in healthy adults, boys and girls.

Time interval	Adults (10 cases)	Boys (12 cases)	Girls (7 cases)
4 hours	—	—	93 ml
4 days	87 ml	—	93 ml
6 days	190 ml	133 ml	—



TABLE 6. *Percentage day-to-day physiological variation of  $V_{VC}$  (see text).*

	Time interval	$V_{VC}$ mean ml	Variation in ml	Variation in %
Adults	4 days	4704	87	1.85
Adults	6 days	4704	190	4.03
Boys	6 days	2712	133	4.9
Girls	4 days	2820	93	3.3

The physiological variation expressed in per cent of the first mean value of  $V_{VC}$  for adults and of the second mean value of  $V_{VC}$  for boys and girls (because the first mean value is significantly higher than the second and the third ones) is shown in Table 6.

The values for  $V_{IC}$  are shown in Table 4. Neither the mean values for the first, the second and the third assay nor the individual differences ( $V_{IC} - V_{ICII}$  and  $V_{ICII} - V_{ICIII}$ ) were statistically significant.

The time interval between the assays varied from 4 hours to 6 days (Tables 1 to 3). The physiological variation during four days was 109 ml for adults, 110 ml for boys and 179 ml for girls; expressed in per cent of the mean first  $V_{IC}$  assay in each group, this is 3.1, 5.6 and 8.8. % respectively.

Thus the physiological variation for the three groups is about 6 % (3 to 9 %).

The mean  $V_{ER}$  values (Table 4) for the three assays do not differ in the adults.

The values of the first assay in the boys and in the girls are perhaps significantly higher than the values of the second and the third assay. One of these differences (mean differences tested against zero) is significant: the first  $V_{ER}$  is larger than the second  $V_{ER}$  at a four-day interval in boys ( $P=0.007$ ).

The physiological variation for each group (excluding groups with six cases or less) is shown in Table 7 and in per cent of the first mean value of  $V_{ER}$  for the adults and the girls, and of the second mean value of  $V_{ER}$  for the boys in Table 8.

TABLE 7. *Physiological variation of the expiratory volume in healthy adults, boys and girls.*

Time interval	Adults (10 cases)	Girls (7 cases)	Boys (12 cases)
4 hours	—	112 ml	—
4 days	77 ml	106 ml	—
6 days	114 ml	—	117 ml

TABLE 8. *Physiological variations for  $V_{ER}$  expressed in per cent.*

	Time interval	Variation in ml	$V_{ER}$ mean ml	Variation in %
Adults	4 days	77	1223	6.3
Adults	6 days	114	1223	9.3
Boys	6 days	117	708	16.5
Girls	4 days	106	895	11.8

TABLE 9. *Day-to-day physiological variations of the lung volumes in healthy adults, boys and girls expressed in percent of the corresponding first assay.*

	$V_{FRC}$	$V_{VC}$	$V_{IC}$	$V_{ER}$	$V_R$	$V_{TLC}$
Adults	7.5	1.9	3.1	6.3	19	5.2
Boys	3.7	4.9	5.6	16.5	27	5.7
Girls	5.8	3.3	8.8	11.8	22	4.5

The physiological variation is from 6 to 17 %.

The mean  $V_R$  values (Table 4) for each group did not differ significantly. Taking only the assays with four-day intervals, the physiological variation was 216 ml for the adults, 145 ml for the boys and 120 ml for the girls; expressed in percent of the first  $V_R$  values the variation is 19, 27 and 22 % respectively.

The  $V_{TLC}$  values (Table 4) also did not differ significantly. The physiological variation during four days is 229 ml for the adults, 191 ml for the boys and 155 ml for the girls.

This percentage is 5.2, 5.7 and 4.5 % respectively.

### Discussion

Analysis of the individual differences and the mean differences reveals the first assay of  $V_{VC}$  is significantly higher than the second for both girls and boys, and the first assay of  $V_{ER}$  is significantly higher than the second for the boys. One could expect the reverse results if there were any influence of training on the values. It seemed to the observers that the children were not "more trained" but "less enthusiastic" after the first assay.

The determination of  $V_{VC}$ ,  $V_{IC}$  and  $V_{ER}$  (and the subsequent calculation of  $V_R$  and  $V_{TLC}$ ) depends upon the cooperation of

the subject. In contrast, determination of  $V_{FRC}$  does not demand any effort from the child. The largest differences observed were for  $V_{ER}$  and  $V_R$  ( $V_{FRC}$  minus  $V_{ER}$ ). The latter difference is primarily due to large variations of  $V_{ER}$  (Table 9).

The physiological variation of the total lung capacity ( $V_{TLC}$ ) is about 5 %. It depends on the variation of both  $V_{IC}$  and  $V_{FRC}$  ( $V_{FRC} + V_{IC} = V_{TLC}$ ). To appreciate the magnitude of the variation of  $V_{TLC}$ , one has to remember that the error of the method for the determination of  $V_{FRC}$  is 2.5 % for adults and boys, 3 % for girls, and that the value for  $V_{IC}$  requires the cooperation of the subject.

Significant differences in the physiological variation of certain of the lung volumes between children and adults were demonstrated for  $V_{VC}$ ,  $V_{IC}$  and  $V_{ER}$ .

The day-to-day variation in those volumes which demand the cooperation of the subject is lesser for the adults than for the children.

Surprisingly, the variation of  $V_{FRC}$  was higher in adults than in children. The explanation is not clear and further study is needed.

### Summary

1. The day-to-day physiological variations of the lung volumes were studied in healthy boys and girls. The results were

compared with the ones obtained in healthy adults.

2. The most striking variation was observed for the values of expiratory reserve

volume which evokes the cooperation of the children and for the values of residual volume (because its calculation is a function of  $V_{ER}$ ).

### References

1. CARLIER, J.: Exploration fonctionnelle respiratoire chez les malades mitraux. *Rev Méd Liège*, 13: 360, 1958.
2. ENGSTRÖM, I., KARLBERG, P., and KRÆPELIEN, S.: Respiratory studies in children. I. Lung volumes in healthy children, 6-14 years of age. *Acta Paediat*, 46: 277, 1956.
3. GEUBELLE, F., and DE RUDDER, P.: Respiratory studies in children. II. Functional residual capacity in healthy children. *Acta Paediat*, in press.
4. — Etudes respiratoires chez l'enfant. I. La capacité vitale et ses composantes. *Acta Paediat Belg*, 13: 233, 1959.
5. PAPPENHEIMER, J. R. et al.: Standardization of definitions and symbols in respiratory physiology. *Fed Proc*, 9: 602, 1950.
6. STRANG, L. B.: Abnormalities of ventilatory capacity in children with asthma and bronchiectasis. *Arch Dis Child*, 35: 224, 1960.

Submitted Jan. 23, 1961

Accepted Jan. 25, 1961

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## Renal Aspects of Acid-Base Control in the Newly Born

### III. Response to Acidifying Drugs

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The renal functions of adults and of newborn infants have been compared in various ways and it has been shown that in many respects the newborn kidney is immature, and McCance & Widdowson (23, 24, 25) have demonstrated that the stability of the "milieu interieur" may be maintained mainly by virtue of the integration of food and growth with renal function. The rôle of adults' kidneys in the control of the hydrogen ion concentration in the internal environment has been fairly well investigated in normal, experimental and pathological states (11, 29, 31, 33, 38, 44), and some account of the natural excretion of acids and bases in the newborn period was given by Widdowson & McCance (43) and McCance & Widdowson (26). No acidifying drugs were given in the last two investigations.

Gordon *et al.* (12) and Fomon *et al.* (10) gave six and eight infants respectively ammonium chloride in divided doses for some days and studied the effects. The infants were of different ages, there were no adult controls, and the results do not strictly apply to the newborn period. Rubin *et al.* (36) made somewhat similar

experiments on premature infants 28 days old. The aim of this present study has been to compare the responses of adults and newborn infants to the administration of single doses of acidifying drugs.

#### Material and Methods

Twenty-four seven day old male babies were studied. The first sample of urine passed in the morning was discarded in each case and the specimens passed for the next four hours were collected under toluene. All the urine was obtained and preserved as described by McCance & Widdowson (22), except that each specimen was exactly timed and saved separately. After this preliminary period of 4 hours, four of the babies were given 2.8 mEq/kg of  $\text{NH}_4\text{Cl}$  (54 mEq/m<sup>2</sup> of body surface) in a single dose and four the same amount of  $\text{CaCl}_2$ . The salt was dissolved in breast milk and given at 1 p.m. immediately before breast feeding. Blood samples were taken by heel prick under paraffin oil before and about 4 hours after giving the acidifying drug. Urine was collected for 8 hours, or as near to this time as the spontaneous voidings of the infants allowed. Eight infants were given 7 mEq of P per day in divided doses on the 5th, 6th and 7th days of their lives. It was given as a mixture of M/7.5  $\text{Na}_2\text{HPO}_4$  and M/7.5  $\text{KH}_2\text{PO}_4$  at pH 7.4. Since the daily intake of P in their mothers milk was of the order of 3.5

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mEq/day, these infants were getting about three times their normal intake of P. On the 7th day urine was collected quantitatively for 24 hours and the pH, titratable acidity, ammonia and phosphorus were determined in it. The results were compared with those collected and described by McCance & Widdowson (26). Eight further babies were given the same phosphate mixture on the 5th, 6th and 7th day of their lives, but on the 7th day, instead of collecting a specimen of urine covering a period of 24 hours, 2.8 mEq of  $\text{NH}_4\text{Cl/kg}$  were administered after a preliminary sample of urine and blood had been collected, and the 8 hours experiment already described was then carried out in exactly the same way.

Eight healthy adults were investigated in a comparable manner. After the collection of a preliminary, timed, urine sample covering a period of 2 hours, blood was taken under paraffin by vein puncture. Four of the adults were given about 1.4 mEq/kg (54 mEq/m<sup>2</sup> body surface) of  $\text{NH}_4\text{Cl}$  and four others took the same amount of  $\text{CaCl}_2$  diluted in water. It was originally intended to give the adults and the infants equal doses per kilogram of body weight, but 2.8 mEq/kg produced nausea and vomiting in the adults. The dosage was therefore equalised per square metre of body surface. Specimens of urine were collected under toluene every two hours for eight hours and a second vein puncture was performed four hours after giving the acidifying drug. Some months later the same eight adults were given 48 mEq of P per day in divided doses at a pH of 7.4 for 48 hours and on the second day the ammonium chloride test was carried out in exactly the same way as before.

Babies seemed to tolerate these doses of  $\text{NH}_4\text{Cl}$  and  $\text{CaCl}_2$  equally well. Neither adults nor newborns suffered any ill effects during the experiment.

The timed specimens of urine passed by the babies during the 8 hours after the acidifying salt had been administered were split up and divided according to their volumes and times into 4 specimens each representing a period of 2 hours. These were separately

analysed and so was the timed specimen passed before the drug was given.

The measurement of pH was made with B.D.H. capillators and the results have been expressed in terms of H ion concentrations so that they could be averaged and treated statistically. Titratable acid was determined by titrating the urine with 0.1 N or 0.01 N NaOH to pH 7.4 with phenol red as indicator. Ammonia was estimated by Conway's method (3); chlorides both in sera and urine by the method of Schales & Schales (37). For phosphorus the method described by Hawk, Oser & Summerson (15) was used. Urinary and serum Na and K were determined with an E.E.L. flame photometer, after suitable dilutions. Serum bicarbonate was determined by Conway's microdiffusion method (3) in No. 2A units using 0.1 ml of serum. The figures for phosphate in mEq were arrived at by taking the valency for phosphate as 1.8. The averages given in the tables are all the means of the findings in 8 subjects, and they are followed by their standard deviations.

### *The basis of comparison*

All those who have worked on the renal function of newborn animals have had to decide upon how their results are to be compared with those of adults (2, 7, 27, 28). Most, but not all (42) of those who have worked on human subjects have made their comparison per square metre of body surface while those who have worked on animals have used body weight. McCance & Widdowson (21) suggested that for some purposes body water would be the most correct basis to employ. This gives results close to those for body weight. Since the method employed may affect the conclusions to be drawn it is most important to use the one which is likely to give results of most value for the purpose in hand. In the present investigation the doses were administered per square metre for the reasons already given. Some of the results are set out in Table 1 in four ways to show how very different they can be made to appear by varying the basis

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TABLE 1. *The renal responses of infants to ammonium chloride compared with those of adults in 4 different ways*

Measurement compared	Basis of comparison			
	m <sup>2</sup>	kg of body wt	basal metabolic rate	as % of potential H ions or of chloride administered
Excretion of:				
Ammonium salts	18	42	20	21
Titratable acid	9	23	11	11
Total acid	14	35	16	17
Chloride	30	76	37	39

X.B. 1. The dose in all cases was 54 mEq NH<sub>4</sub>Cl/m<sup>2</sup>.

2. Only the figures for the infants are given in the Table and each has been calculated on the assumption that the adult value was 100.

3. The basal metabolic rates of infants and of adults were taken to be 162 and 1470 kcal/h respectively—Smith (40), Suzuki (41).

of comparison. It is interesting and perhaps important to note how closely the results expressed per unit of basal metabolism agree with the only direct evidence available, namely the percentage of the dose excreted. Most of the comparisons in this paper have been made on a basis of surface area, but body weight has also been used to present certain aspects. Ultimately it is very much to be hoped that standards of normality will be available for human infants and children of all ages. This will obviate the need for so many comparisons with adults in clinical work, but it is unlikely that such standards of reference will ever be available for all the species of experimental animals likely to be employed.

## Results

### 1. *The changes in the serum*

Table 2 shows the average serum chloride and the total CO<sub>2</sub> in adults and infants before, and 3 to 4 hours after, the administration of ammonium chloride. The total CO<sub>2</sub> was initially a little lower in the infants than in the adults. The effect of the drug was also somewhat greater in infants and it may be assumed that the stimulus to excrete hydrogen ions should have been at least as great or greater.

### 2. *The correction of the acidosis*

Table 3 shows the effect of giving breast-fed human infants 54 mEq of NH<sub>4</sub>Cl or CaCl<sub>2</sub>/m<sup>2</sup> as a single dose and Table 4 what happened when the same treatment was meted out to normal adults. (The dose for adults was much smaller than for infants/kg of body weight.) The infants (*a*) differed considerably more among themselves but raised the average H ion concentration ( $[H^+] \times 10^{-7}$ ) of their urine less rapidly and to a considerably smaller extent than adults; (*b*) had little titratable acid in their preliminary urine and only doubled it after the acidifying drug, whereas the adults had more titratable acid initially and increased it by four times as the  $[H^+] \times 10^{-7}$  of the urine rose; (*c*) excreted nearly as much ammonia as the adults before the drug was given but increased the rate of excretion much less afterwards. Consequently the overall excretion of H ions was less than that of the adults in the preliminary specimens of urine and the response to the acidifying drug much less; (*d*) had only very small amounts of P in the urine at all times; (*e*) excreted the Cl ion less rapidly at all

TABLE 2. *Effect of giving 54 mEq/m<sup>2</sup> NH<sub>4</sub>Cl or CaCl<sub>2</sub> on the chlorides and total CO<sub>2</sub> in the plasma of adults and of breast-fed infants 7 days old with and without neutral phosphate.*

Subjects	Chloride mEq/l		Total CO <sub>2</sub> , mEq/l	
	Before	After	Before	After
Adults	106 ± 2.0	109 ± 2.3	29 ± 1.6	26 ± 2.2
Infants on breast milk alone	109 ± 3.2	113 ± 4.1	24 ± 2.1	19 ± 3.3
Infants on breast milk plus phosphate	109 ± 3.3	115 ± 2.8	25 ± 1.4	21 ± 2.5

times; (f) excreted less sodium and potassium per hour than the adults before the drug was given and at all times afterwards.

Since the amount of titratable acid which can be excreted in the urine is well known to be linked to the amount of buffer substances and particularly phosphates being excreted simultaneously (20, 42) it was thought that the absence of phosphates from the infants' urine at all times might severely limit their capacity to excrete hydrogen ions in the form of titratable acid and consequently their ability to respond to an acidifying drug. Table 5 shows the effect of giving 7 mEq of P, 6.2 mEq of Na and 0.8 mEq of K a day to breast fed infants. The results for normal infants fed on breast milk and

cows' milk have been previously reported (26). The administration of phosphates in this way made little or no difference to the  $[H^+] \times 10^{-7}$  or to the excretion of ammonia over a period of 24 hours, but it raised the excretion of phosphates from 0.75 to 56.9 mEq/kg/24 hr and the titratable acidity by nearly four times. The infants fed on cows' milk preparations had more acid urines and excreted more phosphates, titratable acid and ammonia than either of the two other groups.

Table 6 shows the effect of giving NH<sub>4</sub>Cl as a single dose on the seventh day of life to babies who were being given 7 mEq of P per day on the 5th, 6th and 7th days after birth. The results should be compared with those of the babies who

TABLE 3. *The effect of giving breast-fed infants aged 7 days 54 mEq of NH<sub>4</sub>Cl or CaCl<sub>2</sub>/m<sup>2</sup> as a single dose.*

All the results except those for  $[H^+] \times 10^{-7}$  are given as mEq/m<sup>2</sup>/hr.  
The figures for P = amount excreted in mg  $\times 1.8/31$ .

	Before giving NH <sub>4</sub> Cl or CaCl <sub>2</sub>	Hours after giving NH <sub>4</sub> Cl or CaCl <sub>2</sub>			
		0-2	2-4	4-6	6-8
$[H^+] \times 10^{-7}$	6.2 ± 10.5	12.7 ± 11.5	28.0 ± 28.8	49.8 ± 45.1	58.4 ± 54.5
Titratable acid	0.06 ± 0.05	0.09 ± 0.08	0.12 ± 0.10	0.14 ± 0.10	0.12 ± 0.08
Ammonia	0.40 ± 0.13	0.47 ± 0.17	0.57 ± 0.14	0.59 ± 0.11	0.52 ± 0.17
Ammonia + titratable acid	0.46 ± 0.16	0.55 ± 0.15	0.69 ± 0.22	0.73 ± 0.17	0.65 ± 0.22
Chloride	1.37 ± 0.49	1.90 ± 0.57	3.79 ± 2.53	3.32 ± 1.73	2.85 ± 1.29
P	0.06 ± 0.12	0.08 ± 0.14	0.08 ± 0.18	0.04 ± 0.12	0.04 ± 0.10
Na	1.18 ± 0.55	1.58 ± 0.57	2.87 ± 2.13	2.66 ± 1.73	2.11 ± 1.12
K	0.48 ± 0.20	0.51 ± 0.31	0.82 ± 0.50	0.79 ± 0.43	0.73 ± 0.43



TABLE 4. *The effect of giving normal adults 5 mEq of  $\text{NH}_4\text{Cl}$  or  $\text{CaCl}_2/\text{m}^2$  as a single dose.*

All the results except those for  $[\text{H}^+] \times 10^{-7}$  are expressed as mEq/m<sup>2</sup>/hr.  
The figures for P = amount excreted in mg  $\times 1.8/31$ .

	Before giving $\text{NH}_4\text{Cl}$ or $\text{CaCl}_2$	Hours after giving $\text{NH}_4\text{Cl}$ or $\text{CaCl}_2$			
		0-2	2-4	4-6	6-8
$[\text{H}^+] \times 10^{-7}$	$6.2 \pm 8.2$	$51.2 \pm 59.9$	$137 \pm 83.4$	$142 \pm 35.5$	$150 \pm 38.0$
Titratable acid	$0.26 \pm 0.23$	$0.48 \pm 0.21$	$0.92 \pm 0.22$	$1.01 \pm 0.20$	$0.96 \pm 0.22$
Ammonia	$0.56 \pm 0.23$	$1.29 \pm 0.16$	$1.33 \pm 0.23$	$1.30 \pm 0.32$	$1.34 \pm 0.35$
Ammonia + titratable acid	$0.82 \pm 0.45$	$1.75 \pm 0.33$	$2.25 \pm 0.32$	$2.31 \pm 0.38$	$2.30 \pm 0.69$
Chloride	$3.71 \pm 1.70$	$8.05 \pm 4.90$	$11.88 \pm 4.51$	$8.66 \pm 3.57$	$7.57 \pm 3.66$
P	$0.45 \pm 0.26$	$0.43 \pm 0.23$	$0.72 \pm 0.35$	$0.77 \pm 0.41$	$0.75 \pm 0.27$
Na	$3.21 \pm 1.37$	$6.16 \pm 3.86$	$8.86 \pm 3.68$	$6.11 \pm 3.19$	$5.55 \pm 3.31$
K	$1.34 \pm 0.60$	$1.77 \pm 0.70$	$2.39 \pm 0.58$	$2.05 \pm 0.60$	$1.99 \pm 0.60$

TABLE 5. *Effect of giving 7 mEq P/day in solution at pH 7.4 on urinary pH and on the rates of excretion of inorganic phosphate, titratable acid and ammonia in breast-fed infants 7 days old.*

Treatment	$[\text{H}^+] \times 10^{-7}$	Phosphate $\mu\text{Eq/kg/hr}$	Titratable acid $\mu\text{Eq/kg/hr}$	Titratable acid + ammonia	
				$\mu\text{Eq/kg/hr}$	$\mu\text{Eq/kg/hr}$
Breast milk alone <sup>a</sup>	$11.8 \pm 15.7$	$0.75 \pm 0.46$	$6.3 \pm 4.7$	$23.5 \pm 6.2$	29.8
Breast milk plus phosphate	$11.9 \pm 17.4$	$56.9 \pm 12.4$	$22 \pm 11.4$	$21.5 \pm 4.9$	$43.5 \pm 15.9$
Cow's milk <sup>a</sup>	$16.2 \pm 9.74$	$93 \pm 23.2$	$42 \pm 10.8$	$30 \pm 5.4$	72

<sup>a</sup> Data from McCance & Widdowson (26).

TABLE 6. *The effect of giving  $\text{NH}_4\text{Cl}$  as before to breast-fed infants on the 7th day of their lives. The babies were also being given 7 mEq of P/day on this and on the two previous days.*

All the results except those for  $[\text{H}^+] \times 10^{-7}$  are given as mEq/m<sup>2</sup>/hr.  
The figures for P = the amount excreted in mg  $\times 1.8/31$ .

	Before giving $\text{NH}_4\text{Cl}$	Hours after giving $\text{NH}_4\text{Cl}$			
		0-2	2-4	4-6	6-8
$[\text{H}^+] \times 10^{-7}$	$1.49 \pm 2.46$	$1.78 \pm 1.11$	$3.31 \pm 1.58$	$11.2 \pm 9.0$	$15.3 \pm 11.6$
Titratable acid	$0.19 \pm 0.14$	$0.27 \pm 0.17$	$0.42 \pm 0.22$	$0.47 \pm 0.21$	$0.51 \pm 0.24$
Ammonia	$0.24 \pm 0.09$	$0.42 \pm 0.17$	$0.57 \pm 0.22$	$0.52 \pm 0.21$	$0.42 \pm 0.19$
Ammonia + titratable acid	$0.43 \pm 0.20$	$0.69 \pm 0.32$	$0.99 \pm 0.39$	$0.99 \pm 0.36$	$0.94 \pm 0.31$
Chloride	$0.81 \pm 0.24$	$1.58 \pm 0.45$	$2.17 \pm 0.90$	$2.12 \pm 1.18$	$1.98 \pm 1.08$
P	$0.63 \pm 0.27$	$1.02 \pm 0.32$	$0.85 \pm 0.42$	$0.80 \pm 0.38$	$0.89 \pm 0.52$
Na	$1.28 \pm 0.40$	$2.31 \pm 0.66$	$2.30 \pm 1.11$	$2.02 \pm 1.21$	$1.80 \pm 1.15$
K	$0.50 \pm 0.25$	$0.68 \pm 0.26$	$0.61 \pm 0.29$	$0.65 \pm 0.27$	$0.74 \pm 0.40$

TABLE 7. *The effect of giving  $\text{NH}_4\text{Cl}$  in adults as before, who were also taking 48 mEq of P in divided doses on the same and on the previous day.*

All the results except those for  $[\text{H}^+] \times 10^{-7}$  are given as mEq/m<sup>2</sup>/hr.  
The figures P = the amount excreted in mg  $\times 1.8/31$ .

	Before giving $\text{NH}_4\text{Cl}$	Hours after giving $\text{NH}_4\text{Cl}$			
		0-2	2-4	4-6	6-8
$[\text{H}^+] \times 10^{-7}$	$7.76 \pm 8.75$	$39.9 \pm 33.1$	$148 \pm 88.9$	$85.2 \pm 40.2$	$63.4 \pm 52.1$
Titratable acid	$0.38 \pm 0.23$	$0.97 \pm 0.38$	$1.84 \pm 0.43$	$1.99 \pm 0.43$	$1.98 \pm 0.50$
Ammonia	$0.59 \pm 0.14$	$1.44 \pm 0.23$	$1.37 \pm 0.25$	$1.41 \pm 0.29$	$1.28 \pm 0.32$
Ammonia + titratable acid	$0.97 \pm 0.32$	$2.41 \pm 0.52$	$3.21 \pm 0.61$	$3.40 \pm 0.68$	$3.26 \pm 0.78$
Chloride	$4.13 \pm 1.50$	$8.04 \pm 2.85$	$8.84 \pm 2.61$	$8.51 \pm 1.86$	$6.71 \pm 1.39$
P	$0.86 \pm 0.47$	$1.43 \pm 1.10$	$2.44 \pm 0.95$	$3.00 \pm 0.98$	$3.52 \pm 1.51$
Na	$3.84 \pm 1.37$	$6.40 \pm 2.51$	$6.93 \pm 2.29$	$6.89 \pm 0.81$	$5.34 \pm 1.71$
K	$1.54 \pm 0.80$	$1.98 \pm 0.65$	$2.29 \pm 0.68$	$3.07 \pm 1.35$	$3.23 \pm 1.03$

were not given phosphates (Table 3) and with those of the adults in Tables 4 and 7. Table 7 shows the effect of giving 48 mEq of phosphorus per day for two days as a neutral solution to adults and 54 mEq of  $\text{NH}_4\text{Cl}/\text{m}^2$  on the second of these two days.

The babies given phosphates plus  $\text{NH}_4\text{Cl}$  (a) excreted 0.63 to 1.02 mEq of P/m<sup>2</sup>/hr throughout the test; (b) raised the  $[\text{H}^+] \times 10^{-7}$  of their urines no more, indeed rather less, than those not given phosphates, but (c) excreted more titratable acid before and considerably more after being given the ammonium chloride; (d) excreted less ammonia before and slightly less after the acidifying drug. Owing to the increased excretion of titratable acid, however, these infants got rid of more of the H ions generated by the drug than those not taking phosphates in spite of the fact that the  $[\text{H}^+] \times 10^{-7}$  of their urines was never quite so high but (e) they excreted less chloride ions; (f) their excretion of sodium and potassium was about the same.

Although the dose was relatively much smaller, giving the phosphates to the adults increased by 2-4 times the amounts

of P being excreted in the urine. It made no appreciable difference to the rate at which these adults were able to raise the  $[\text{H}^+] \times 10^{-7}$  of their urine after  $\text{NH}_4\text{Cl}$  and consequently it greatly increased the excretion of titratable acid. It made little or no difference to the rate of excretion of ammonia at any time but, as in the babies, it materially increased the elimination of the H ions generated by the drug and, also, as in the babies, it reduced the excretion of chloride ions.

Table 8 gives four ways in which the differences between the ability of the adult and the newly born to correct the acidosis may be summarised. It shows in order the maximum rate of excretion of titratable acid + ammonia and the maximum H ion "clearance" index, both measured over 2 hour periods. The H ion clearance is the most sensitive method hitherto devised of assessing the ability of a person to maintain systemic neutrality. It was worked out by Elkinton & McCance (Elkinton, Huth, Webster & McCance (9)). The clearance "index" is defined as the mEq of titratable acid + ammonia excreted per min per  $1.73 \text{ m}^2 \times \text{plasma bicarbonate}$

TABLE 8. *The greatest rate of excretion of H ions + NH<sub>3</sub> and the highest H ion clearance index (both measured over two hour periods) and the percentage of the dose of potential H ions and of chlorides excreted within 8 hours by adults and infants.*

Measurement	Infants	Adults
Maximum rate of excretion of H ions mEq/1.73 m <sup>2</sup> /hr without phosphate pretreatment	1.09	4.00
Maximum H ion clearance index without phosphate pretreatment	0.35	1.70
Maximum rate of excretion of H ions mEq/1.73 m <sup>2</sup> /hr after the administration of phosphate	1.70	6.00
Maximum H ion clearance index after the administration of phosphate	0.57	2.50
Percentage of the H ion excreted in 8 hours		
(a) Without administering phosphates	3.4 ± 1.8	19.7 ± 6.9
(b) After administering phosphates	8.8 ± 2.8	31.0 ± 6.5
Percentage of the Cl ions excreted in 8 hours		
(a) Without administering phosphates	32.0 ± 17.3	79.9 ± 33.7
(b) After administering phosphates	21.2 ± 12.7	55.5 ± 23.4

(in mEq). Table 8 also shows the percentage of the dose of potential H ions generated by the drug excreted at each age within 8 hours, and the percentage of the chloride ions. In each case the rate of excretion before the drug was given has been taken as the base line and deducted from the rates found over the next 8 hours.

In all four parameters the infants made a poor showing as compared with adults, but it must be recalled that had the dosages and results been expressed per kg of body weight the infants would have received a larger dose than the adults but they would have made a much better showing.

### 3. *The total response to ammonium chloride*

The average cation-anion relationship of the urine of the unmedicated breast-fed infants is shown in Fig. 1a. It is known from the work of Slater (39) on 18 other breast-fed infants, that on the 7th day of their lives infants absorb about 95  $\mu$ Eq/kg/hr of potassium, 100 of sodium, 107 of

chloride and 49 of phosphorus, and it may be taken that the babies in the present investigation were absorbing about the same. The serum-tissue-renal relationships were causing the excretion of 145  $\mu$ Eq/kg/hr of (measured) cations, mostly balanced by chlorides and there were only traces of phosphates in the urine. The administration of the phosphates increased the intake of total cations by 84  $\mu$ Eq/kg/hr for the previous 48 hours as shown in Fig. 1b. This was not a large addition, for the food had been providing about 195  $\mu$ Eq/kg/hr and the addition provoked almost no change in the excretion of total cations (see Fig. 1c). The rate of excretion of phosphates went up—if only to about half that of the additional intake—and this led to the readjustment between titratable acid and ammonia which has already been noted—but since there was only a trifling increase in the total excretion of cations the rate of excretion of chlorides necessarily fell. This may be regarded as an example of the ion antagonism discussed by Rapoport & West

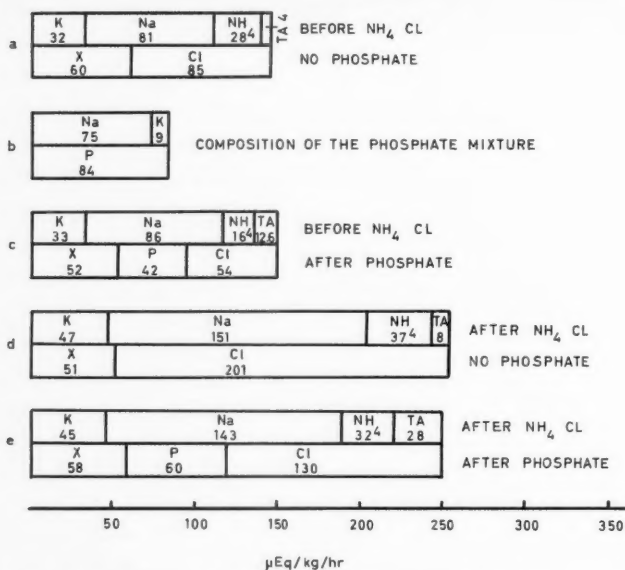


Fig. 1. The effect of giving  $\text{NH}_4\text{Cl}$  without and with pretreatment with neutral phosphates on the cation-anion balances of the urine in infants. The measurements shown in blocks *a* and *c* refer to the preliminary periods, and those in blocks *d* and *e* cover the 8 hours after the drugs had been given. *b* shows the composition of the neutral phosphates administered. ( $\times$  = undetermined anions.)

(34), but the results of Bank & Schwartz (1) should also be considered. These last authors found that the infusion of neutral sodium phosphate to sodium depleted dogs led to the preferential excretion of the phosphate, much titratable acid and urines with a very low pH.

Giving the ammonium chloride without the preliminary administration of phosphates created an internal acidosis (see Table 2). Fig. 1*d* shows that this raised the excretion of titratable acid + ammonia from 32 to 45  $\mu\text{Eq/kg/hr}$ . This may not seem much and it was only a fraction of the total response (compare Fig. 1*a*) which included a large rise in the excretion of Na and a smaller one in that of K. The rate of cation excretion rose from 145

to 250  $\mu\text{Eq/kg/hr}$  and the whole of this was balanced by chlorides.

The exhibition of the neutral phosphates made little difference to the internal acidosis created by the ammonium chloride (Table 2) nor did it make any difference to the total cation response of the kidney (Fig. 1*d* and *e*). The phosphates, however, considerably increased the rate of excretion of titratable acid + ammonia and produced a readjustment between them as it had done when no  $\text{NH}_4\text{Cl}$  had been given (Fig. 1*d* and *e*, and *a* and *c*). The rate of excretion of phosphates was considerable although again less than the additional intake (Fig. 1*a* and *c*) and again, since giving the phosphates did not increase the rate of excretion of cations,

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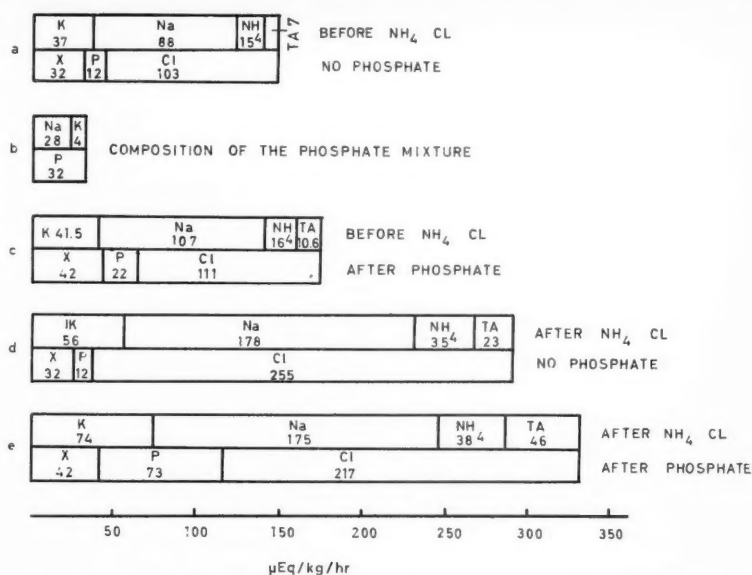


Fig. 2. The effect of giving  $\text{NH}_4\text{Cl}$  without and with pretreatment with neutral phosphates on the cation-anion balances of the urine in adults. The measurements shown in blocks a and c refer to the preliminary periods, and those in blocks d and e cover the 8 hours after the drugs had been given. b shows the composition of the neutral phosphates administered. (x = undetermined anions)

the rate of excretion of chlorides necessarily fell.

The average anion-cation relationships of the urine of the normal adults are shown in Fig. 2a. Without having been given phosphates or ammonium chloride the adults excreted 148  $\mu\text{Eq}$  of measured cations per hour, which was close to the rate in infants, although there were differences in the ammonia-titratable acid relationships. These cations were balanced by rather more chlorides than in the infants and by phosphates.

It cannot be assumed the rates of intake of sodium, chloride, potassium and phosphorus were equal to the measured rates of excretion, for the latter are subject to considerable diurnal variations, but all the

experiments were done at the same time of day to minimise this variable. The administration of the phosphates increased the intake of total cations considerably less than in infants (see Fig. 1b). Nevertheless it raised the excretion of titratable acid and of sodium, and almost doubled the excretion of phosphorus (Fig. 2c).

The internal acidosis provoked by ammonium chloride without phosphates raised the excretion of titratable acid and ammonia, increased the excretion of potassium and considerably increased that of sodium. This last was almost entirely balanced by chloride and there was no increase in the excretion of phosphorus (Fig. 2a and d). This was a materially greater total response, however, than the

ammonium chloride produced in the babies (compare Fig. 1 *d* and 2 *d*). After giving the neutral phosphates to the adults as well as ammonium chloride there was the usual increase in the excretion of total cations (compare Fig. 2 *c* and 2 *e*). The increased excretion of phosphorus was roughly the same as in the infants and, although the excretion of chlorides more than doubled, the increase was not so great as it had been when phosphates had not been given (Fig. 2 *a* and *d*, and *c* and *e*). Thus the total response to the internal acidosis in infants was fundamentally the same as in adults and consisted of an increase in the output of titratable acid and to some extent of ammonia, but also a great increase in the output of sodium and chloride. The administration and excretion of the phosphates did not make any difference to the output of total cations by the babies and not a great one to the output in adults, and consequently the increased excretion of phosphates led to the excretion of less chlorides, at both ages.

### Discussion

The administration of neutral phosphates to infants 7 days old resulted in a considerable increase in the elimination of free H ions, without any significant decrease in the output of ammonium ions. It is possible that even before any acidifying drug was administered the capacity of the infants to eliminate H ions at the existing concentration of total CO<sub>2</sub> in the plasma was limited to some extent by the absence of enough urinary buffers. This at all events is the assumption usually made about patients with renal acidosis (18, 44).

It may be instructive to regard the findings in the serum and urine of the newborn infant as originating in the same way as those in a case of incomplete renal acidosis. Since, however, it was pointed out by Schiess *et al.* (38) that the administration of neutral phosphate may have the above effect in normal adults the difference between them and the newborn must be regarded as a relative rather than an absolute one.

These present experiments have differed in two respects from any of the previous ones carried out on human infants. Thus (1) they were based on the effects of administering single doses of the acidifying drugs and (2) the infants were breast-fed and therefore excreting little or no inorganic phosphates. The present experiments, therefore, were more like those of Cort & McCance (4) on newborn puppies than those of Gordon *et al.* (12) or of Fomon *et al.* (10) on infants.

After the administration of the acidifying drugs the average  $[H^+] \times 10^{-7}$  of the infants urines rose less quickly and completely than it did in adults. In other words the infants were unable within the time limits of these experiments to establish as great an H ion gradient between plasma and urine. This is probably true of other newborn animals but puppies have been found more able than human infants (4) and piglets less able (14). Without enquiring too closely into the mechanisms involved the results again recall those found in cases of renal acidosis. The infants studied by Gordon *et al.* (12) excreted 24 hour urines with pH values as low as 4.6 after ammonium chloride but they had been longer out of the uterus than the present series, their serum bi-

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carbonates became much more abnormal, they were being fed on cows' milk and the drugs were given for several days. Even in those experiments, however, some of the infants did not lower the pH of their urine (12, 17) as much as adults would have done.

The lack of phosphates in the urine of newborn infants severely limited the excretion of titratable acid at all times. However, even after the administration of neutral phosphates plus  $\text{NH}_4\text{Cl}$  the average rates of titratable acid excretion did not reach adult levels, partly because the infants failed to raise the  $[\text{H}^+] \times 10^{-7}$  of their urines as much as the adults did. These results, therefore, confirmed a prediction of Dean & McCance (6). The results after administering phosphates as well as  $\text{NH}_4\text{Cl}$  were not unlike those obtained by Pitts & Lotspeich (32) on acidotic dogs.

Most previous experiments, including those of Cort & McCance (4) (see also Robinson (35)) have turned on the ability of the newborn subject to excrete ammonium ions and the general conclusion has been that the defect relative to adults may be considerable. This is probably correct so far as puppies are concerned. The present experiments have brought out new features in man. Owing to the paucity of buffer substances in the urine, the human infant before birth and, if breast-fed, after birth, must rely upon ammonia for the greater part of the H ion elimination in the urine (20). McCance & Widdowson (26) found that at 7 days of age the normal rate of excretion of ammonium per kg of body weight was equal to that of adults and the present results after  $\text{NH}_4\text{Cl}$  bears this out if they are looked at

per kg of body weight instead of per unit of surface area. It is possible that the ammonia production of these infants was reasonably good and being utilised almost to capacity before the drugs were given, and that this accounts in part for the small increase afterwards. From the data of other investigators, however, it seems that the ability to increase the excretion of ammonia during an acidosis is not as well developed in breast-fed infants as it is in those fed on cows' milk (10). Possibly the availability of ammonia precursors (30), or the development of the necessary enzymatic processes (5, 16) may also be limiting factors.

To judge by these experiments the newborn breast-fed infant is in a state of mild acidosis as indicated by its serum bicarbonate and its response to the administration of a solution of neutral phosphates. The excretion of H ions generated by the administration of ammonium or calcium chloride is limited by (a) the failure to raise the  $[\text{H}^+] \times 10^{-7}$  of the urine so rapidly or so fully as adults and (b) the paucity of urinary buffers. Even when this was completely rectified the rate of H ion excretion (in all forms) was still only two-thirds that of adults, and certainly not so good at corresponding levels of plasma "total  $\text{CO}_2$ " or following a similar fall from the normal steady state. This all suggests that the ceiling for the production of H ions by the infants' kidney is lower than it is in adults.

Cows' milk provides the infant with more acid to excrete than breast milk (probably mostly sulphates) but also provides buffer substances in the shape of phosphates with which to excrete it as titratable acidity. In healthy newborn infants



the two effects roughly cancel each other out but the infant on cows' milk is usually slightly the more acidosed than the one on breast milk (13, 19). Owing to the continued ingestion of cows' milk, however, the kidneys of infants so fed may become able to excrete H ions better than those of breast-fed infants, for as early as the third month their hydrogen ion clearance indices may reach the adult levels (mean 2.07; range 1.4–3.4) (9) after the administration of acidifying drugs, while those of breast-fed infants of a similar age, calculated from the data of Fomon *et al.* (10) are still very much lower with a mean of 1.01 and a range of 0.34–1.40.

It is not usual to consider what has been termed the "total response" to ammonium chloride, although others have spoken rather teleologically of the increase in ammonia production "sparing" the excretion of fixed base. It is suggested that the excretion of sodium and potassium is part of the response of the organism to a fall in the pH of its plasma and tissues, and that it represents the excretion of sodium and potassium which have been rendered temporarily redundant by the operation of the buffering systems in all parts of the body (8). The pattern of all these responses may be found to differ from the newborn animal of one species to that of another according to their states of development at birth (14).

### Summary

1. When a solution of sodium and potassium phosphates at pH 7.4 were administered to normal breast-fed infants aged 5, 6 and 7 days, they excreted considerably more H ions (in the form of titratable acid).

2. After the administration of a single dose of an acidifying drug normal breast-fed infants 7 days old (a) raised the  $[H^+] \times 10^{-7}$  of their urine less rapidly and completely than adults, (b) excreted less of the H ions generated by the drugs but (c) excreted almost as much ammonia/kg of body weight.

3. The administration of neutral phosphates to adults (a) made no difference to the rate and extent of the rise of  $[H^+] \times 10^{-7}$  after ammonium chloride, (b) increased the excretion of H ions in the form of titratable acid without reducing the excretion of ammonium ions. After giving phosphates to the infants they (a) maintained the  $[H^+] \times 10^{-7}$  of the urine at a lower level, (b) increased the excretion of free H ions but (c) reduced the excretion of ammonia and consequently (d) did not increase the excretion of free H ions + ammonia so much as adults.

4. The normal breast-fed infant appears to be handicapped in dealing with a sudden acidosis by (a) an inability to raise the  $[H^+] \times 10^{-7}$  of the urine rapidly and completely, (b) an absence of urinary buffers, (c) an overall failure to excrete H ions so rapidly as adults. This may amount to a general deficiency on the part of the kidney to produce the necessary H ions to be excreted.

### Acknowledgements

The authors are very grateful to Mr. D. Gairdner for allowing them to study these infants and to the Matron and staff of the Maternity Hospital for their co-operation. Miss P. Hennessy and Dr. E. M. Widdowson have both—each in their own way—given valuable assistance.

## References

1. BANK, N., and SCHWARTZ, W. B.: The influence of anion penetrating ability on urinary acidification and the excretion of titratable acid. *J Clin Invest*, 39: 1516, 1960.
2. BARNETT, H. L., and VESTERDAL, J.: The physiologic and clinical significance of immaturity of kidney function in young infants. *J Pediatr*, 42: 99, 1953.
3. CONWAY, E. J.: Microdiffusion Analysis and Volumetric Error. 4th ed. London: Crosby, Lockwood & Son.
4. CORT, J. H., and McCANCE, R. A.: The renal response of puppies to an acidosis. *J Physiol*, 124: 358, 1954.
5. DAVIES, B. M. A., and YUDKIN, J.: Studies in biochemical adaptation. The origin of urinary ammonia as indicated by the effect of chronic acidosis and alkalosis on some renal enzymes in the rat. *Biochem J*, 52: 407, 1952.
6. DEAN, R. F. A., and McCANCE, R. A.: Phosphate clearances in infants and adults. *J Physiol*, 107: 182, 1948.
7. EDELMANN, C. M., JR., and BARNETT, H. L.: Role of the kidney in water metabolism in young infants. *J Pediatr*, 56: 154, 1960.
8. ELKINTON, J. R.: Whole body buffers in the regulation of acid-base equilibrium. In *Essays in Metabolism*. Boston, Toronto: Little Brown & Co.
9. ELKINTON, J. R., HUTH, E. J., WEBSTER, G. D., JR., and McCANCE, R. A.: The renal excretion of hydrogen ion in renal tubular acidosis. I. Quantitative assessment of the response to ammonium chloride as an acid load. *Amer J Med*, 19: 554, 1960.
10. FOMON, S. J., HARRIS, D. M., and JENSEN, R. L.: Acidification of the urine by infants fed human milk and whole cow's milk. *Pediatrics*, 23: 113, 1959.
11. GILMAN, A., and BRAZEAU, P.: The role of the kidney in the regulation of acid-base metabolism. *Amer J Med*, 15: 763, 1953.
12. GORDON, H. H., McNAMARA, H., and BENJAMIN, H. R.: The response of young infants to ingestion of ammonium chloride. *Pediatrics*, 2: 290, 1948.
13. GYÖRGY, P., KNAPPES, F., and KRUSE, F.: Das Säurebasengleichgewicht im Blut, mit besonderer Berücksichtigung des Kindesalters. I. H-ionenkonzentration und  $\text{CO}_2$ gehalt. *Z Kinderheilk*, 41: 700, 1926.
14. HATEMI, N., and McCANCE, R. A.: The response of piglets to ammonium chloride. To be published in *J Physiol*.
15. HAWK, P. B., OSER, B. L., and SUMMERSON, W. H.: *Practical Physiological Chemistry*. 13th ed. London: J. & A. Churchill. 1954.
16. HINES, B. E., and McCANCE, R. A.: Ammonia formation from glutamine by kidney slices from adult and newborn animals. *J Physiol*, 124: 8, 1948.
17. HOFFMAN, W. S., PARMELEE, A. H., and GROSSMAN, A. Mechanism of production of acidosis in premature infants by protein milk. *Amer J Dis Child*, 75: 637, 1948.
18. LATNER, A. L., and BURNARD, E. D.: Idiopathic hyperchloremic acidosis of infants (Nephrocalcinosis infantum). Observations on the site and nature of the lesion. *Quart J Med*, 19: 285, 1950.
19. MARPLES, E., and LIPPARD, V. W.: Acid-base balance of newborn infants. III. Influence of cow's milk on the acid-base balance of the blood of newborn infants. *Amer J Dis Child*, 45: 294, 1933.
20. McCANCE, R. A., and VON FINCK, M. A.: The titratable acidity, pH, ammonia and phosphates in the urines of very young infants. *Arch Dis Child*, 22: 200, 1947.
21. McCANCE, R. A., and WIDDOWSON, E. M.: The correct physiological basis on which to compare infant and adult renal function. *Lancet* 11: 860, 1952.
22. — Renal function before birth. *Proc Roy Soc B*, 141: 488, 1953.
23. — Metabolism, growth and renal function of piglets in the first days of life. *Arch Dis Child*, 133: 373, 1956.
24. — New thoughts on renal function in the early days of life. *Brit Med Bull*, 13: 3, 1957.
25. — The response of the newborn piglet to an excess of potassium. *J Physiol*, 141: 88, 1958.
26. — Renal aspects of acid-base control in the newly born. I. Natural development. *Acta Paediat*, 49: 409, 1960.
27. McCANCE, R. A., and YOUNG, W. F.: The secretion of urine by newborn infants. *J Physiol*, 99: 265, 1941.
28. METCOFF, J.: Some aspects of renal structure and function concerned with regulation of body fluids. *Pediat Clin N Amer*, 6: No. 1, 1959.
29. ORLOFF, J.: The role of the kidney in the regulation of acid-base balance. *Yale J Biol Med*, 29: 211, 1956.
30. ORLOFF, J., and BERLINER, R. W.: The mechanism of the excretion of ammonia in the dog. *J Clin Invest*, 35: 223, 1956.
31. PITTS, R. F.: Acid-base regulation by the kidneys. *Amer J Med*, 9: 356, 1950.
32. PITTS, R. F., and LOTSPEICH, W. D.: Factors governing the rate of excretion of titra-

- table acid in the dog. *Amer J Physiol*, 147: 481, 1946.
33. PITTS, R. F., LOTSPEICH, W. D., SCHIESS, W. A., and AYER, J. L.: The renal regulation of acid-base balance in man. I. The nature of the mechanism for acidifying the urine. *J Clin Invest*, 27: 48, 1948.
  34. RAPOPORT, S., and WEST, C. D.: Ionic antagonism. Effect of various anions on chloride excretion during osmotic diuresis in the dog. *Amer J Physiol*, 162: 668, 1950.
  35. ROBINSON, J. R.: Ammonia formation by surviving kidney slices without specific substrates. *J Physiol*, 124: 1, 1954.
  36. RUBEN, B. L., CALCAGNO, P. L., RUBIN, M. I., and WEINTRAUB, M. D.: Renal defense response to induced acidosis in premature infants (ammonia production and titratable acid excretion). *Amer J Dis Child*, 92: 513, 1956.
  37. SCHALES, O., and SCHALES, S. S.: A simple and accurate method for the determination of chloride in biological fluids. *J Biol Chem*, 140: 879, 1941.
  38. SCHIESS, W. A., AYER, J. L., LOTSPEICH, W. D., and PITTS, R. F.: The renal regulation of acid-base balance in man. II. Factors affecting the excretion of titratable acid by the normal human subject. *J Clin Invest*, 27: 57, 1948.
  39. SLATER, J. E.: Retentions of nitrogen and minerals by babies one week old. *Brit J Nutr*, 1961.
  40. SMITH, C. A.: The Physiology of the Newborn Infant. 3rd ed. Oxford: Blackwell Scientific Publications, 1959.
  41. SUZUKI, S.: Basal metabolism in the Japanese population. *World Rev Nutr Dietetics* Chap. 5: 105, 1960.
  42. WEBER, H.: Über die Beziehung zwischen Phosphat und Säureausscheidung im Harn bei gesunden und kranken Kindern. *Helv Paediat Acta*, 15: 186, 1960.
  43. WIDDOWSON, E. M., and MCCANCE, R. A.: The development of acid-base control. *Ciba Foundation Colloquia on Ageing*, 4: 209, 1958.
  44. WRONG, O., and DAVIES, H. E. F.: The excretion of acid in renal disease. *Quart J Med*, 28: 259, 1959.

Submitted Dec. 13, 1960

Accepted Jan. 30, 1961

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## Preliminary Experiences with the Spitz-Holter Ventriculo-Caval Shunt in the Treatment of Hydrocephalus<sup>1</sup>

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Since the beginning of the present century surgeons have tried to solve the problem of treating communicating hydrocephalus, but only recently have the results been promising. In the last few years a large number of cases have been operated on utilizing plastic tubing for drainage of cerebrospinal fluid (CSF) into the abdominal or pleural cavities or into the ureter. The attempts to drain this fluid directly into the circulating blood have been unsuccessful due to thrombosis. By using valves allowing only uni-directional flow of CSF into the blood, the number of successful cases has increased. Such a valve, designed by Spitz and Holter, has been used in America and England with promising results. We have used this technique in five cases of progressive infantile hydrocephalus and in one case of intracranial hypertension due to diffuse melanosis of the meninges. The preliminary results have been encouraging.

### History

The first attempt to relieve hydrocephalus by operation was the simple drainage of CSF to the exterior of the skull but these patients soon died from infection. Drainage of CSF back to the blood was suggested by Gärtner as early as 1895. In 1908 Payr (18) reported operations in three cases where the lateral ventricle was anastomosed to the superior sagittal sinus utilizing a free segment of vein. However, all patients died within four months of the operation. Later Payr (1911) reported other ventriculo-venous shunt operations where connection between the lateral ventricle and the internal jugular vein was made. Excellent results were obtained in three out of eight patients. For a comprehensive review of the earlier operative methods in the treatment of hydrocephalus see Davidoff (1929).

Decreased production of CSF by excision of the choroid plexus was first suggested by Dandy in 1919 and it was further modified and improved by Hyndman (1946) and Scarff (1952). The latter reported twenty-five good results out of thirty-nine operated cases with observation times of more than six years.

Ingraham *et al.*, (1948) were the first to report the use of plastic tubing to drain CSF into the circulating blood of experimental animals. In every instance blood refluxed into the tube and clotted. They concluded that a valve allowing only uni-directional

<sup>1</sup> Presented at the 14th annual meeting of the Scandinavian Society of Neurosurgery, Stockholm, September 1959.

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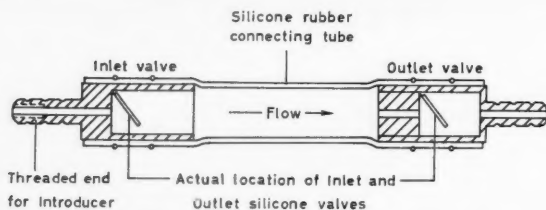


Fig. 1. Simplified drawing of Holter valve to illustrate the principles of construction.

flow of CSF into the blood would be necessary. The first really promising clinical results were presented by Cone *et al.* (1949) using lumbo-peritoneal shunts and Spitz & Koop (1952) reported seventy-three per cent success with this technique. Matson (1949) introduced the lumbo-ureteral shunt and of forty-seven patients thirty-six good results were reported (15). The disadvantages of the Matson procedure are the sacrifice of one kidney and the dangers of electrolyte losses and infection. Another technique, developed by Hakim (1955), consists of shunting CSF from the lateral ventricle to the extradural space of the cervical and thoracic regions where large amounts of fluid can be absorbed. In Scandinavia, Andersson & Bohm (1958) have reported their experience using the lumbo-peritoneal shunt technique and in their cases the tip of the catheter was connected to a specially constructed teflon button. Of nine operated cases three showed good results.

The first use of a valve in ventriculo-venous shunts as suggested by the experiments of Ingraham *et al.* was reported by Nulsen & Spitz (1952). They used a system consisting of a rubber subcutaneous pump connecting two ball valves. This case was operated on in 1949 and the shunt was functioning well at the time of their initial report in 1952. Further modifications and improvements were made by Holter in 1952 (Holter (1959), see also Menab (1958)). Another type of valve has been successfully used by Pudenz *et al.* (1957). Here the flow-controlling valve is situated in the tip of a catheter going down the superior vena cava to the right atrium.

### The Holter valve

The Holter valve consists of a pair of "fishmouth"-type valves connected in series by a short length of silicone rubber tubing. This valve allows only unidirectional flow of fluid and is designed to open at two different pressures of about ten and fifty mm of water respectively. It is about 5 cm in length and 6 mm in diameter (Fig. 1).

The valve system is placed under the scalp in the mastoid region. A proximal catheter penetrates the brain so that the tip will lie freely in the lateral ventricle. Another catheter attached to the distal valve is tied into the internal jugular vein and the tip of this catheter should lie freely in the right atrium.

The valve mechanism lies directly under the scalp and by compressing the rubber cylinder connecting the two valves fluid can be pumped at any time to confirm that the system is patent. This is one of the great advantages of the Holter valve. The site of a possible block can also be determined. Compression of the valve mechanism without refilling denotes a block in the ventricle. If the valve is not compressible there is obstruction on the venous side.

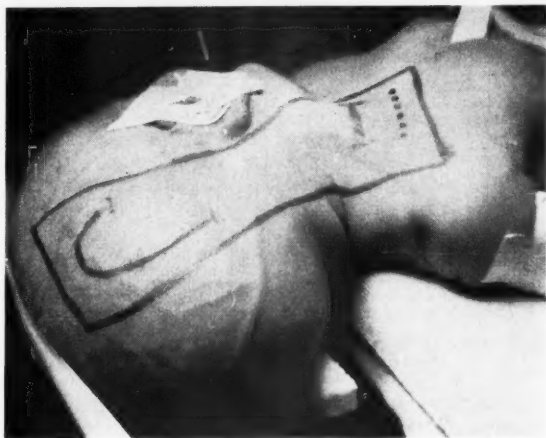


Fig. 2.

### *Operative technique*

A preoperative roentgenogram is taken in order to estimate the approximate length of the distal, cardiac catheter. This length is measured from the point where the catheter is tied into the jugular vein down to 1 cm above the carina (junction of superior vena cava and right atrium). About 10 per cent is deducted due to magnification.

The child is placed on the operating table in the supine position with the head turned to the left exposing the right temporal region and stretching the right side of the neck (Fig. 2). A curved incision is made in the right posterior temporal region and the flap is retracted. A burr hole for the ventricular catheter is placed so that the valve does not extend down into the neck area. A groove is made in the bone to keep the valve in place. The proximal, ventricular catheter is inserted into the lateral ventricle using a stylet. An incision is made in the neck, and the internal jugular vein exposed by blunt dissection through the sternomastoid muscle. By the use of a special introducer the valve

with its cardiac catheter already attached is passed through the cervical incision up under the skin to its position in the groove in the mastoid region where it is fixed with a steel wire. The ventricular catheter is now attached to the proximal valve of the pump. The jugular vein is opened and the cardiac catheter passed down the vena cava. (Beware of air embolism!) The proper position of the tip of the catheter may be checked by a roentgenogram, by fluoroscopy or by electrocardiography (23). In the latter case a conducting wire is passed through the caval catheter so that its tip lies just above the distal opening of the tubing. This wire and one arm are connected to the electrocardiograph, forming an ECG lead. Maximal size of the P-deflection indicates that the tip of the catheter is situated near the centre of the auricle.

At the site where the cardiac catheter passes into the jugular vein, the catheter is divided and threaded over a special plastic connector, which can then be firmly tied into the jugular vein without risk of obstructing the catheter.

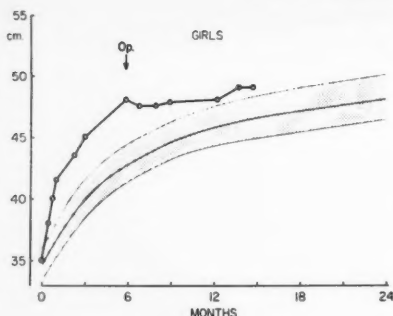


Fig. 3 Case 1. Ordinate: head circumference in cm. Abscissa: age in months.

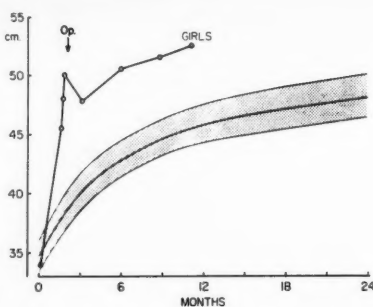


Fig. 4. Case 2. Ordinate: head circumference in cm. Abscissa: age in months.

### Material and Methods

Our material comprises five cases of communicating hydrocephalus, two of which had been previously operated for a meningocele. In one case an Arnold-Chiari malformation was diagnosed. All cases were examined by pre- and postoperative measurements of head circumference, pneumography (postoperatively with small amounts of oxygen), recording of the ventricular fluid pressure (VFP) and mental and physical examination by pediatricians.

In the diagrams illustrating the increase in head circumference the shaded area represents the distribution in 80 per cent (between 10 and 90 per cent) of a study of white infants of North European descent living in or near Boston (24).

The VFP was recorded during 20 to 30 minutes using a technique described by Lundberg (13). In this technique the ventricle is punctured with the cannula connected to the pressure chamber of a strain gauge transducer. Escape of fluid during the procedure is thus avoided. Care was taken to obtain records during periods, when the child was calm, if necessary during feeding.

The last observations included in the follow-up were made in November 1959.

### Case Reports

*Case 1:* G.-M. S. Born May 19, 1958. Immediately after birth vomiting and

rapidly increasing head circumference of unknown etiology (Fig. 3). July 23, 1958, encephalography: communicating hydrocephalus. November 10, 1958, operation: Spitz-Holter ventriculo-caval shunt. No complications. Control examination August 10, 1959: pediatric examination, normal development. Lumbar encephalography, slight increase in brain substance (measured at frontal lobe); VFP 3 to 5 mm Hg under pentothal anesthesia.

*Case 2:* K. G. Born September 19, 1958. During first weeks of life rapid increase in head circumference of unknown etiology (Fig. 4). November 14, mean VFP about 20 mm Hg (Fig. 5). Encephalography November 17, 1958: communicating hydrocephalus. November 19, 1958: Spitz-Holter ventriculo-caval shunt. Fever of unknown origin after a few weeks without local signs of infection. August 1959, lumbar encephalography and recording of VFP: increase in brain substance; mean VFP about 3 mm Hg (Fig. 5). October 1959, intermittent signs of increased intracranial pressure. October 21, 1959, reoperation. Free passage in both catheters, the distal catheter checked by injection of radio-opaque material; valve functioning well. November 8, 1959, signs of acute increase of intracranial pressure. Reoperation: obstruction in proximal catheter relieved, catheter too long. Postoperatively no complications, valve functioning well. Pediatric examination: normal development.



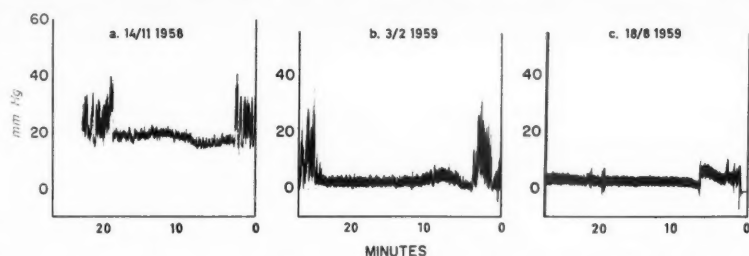


Fig. 5. Case 2. Ventricular fluid pressure before (a) and after (b, c) shunt operation. Large deflections in Figs a and b produced by crying. (When the child was quiet the records always showed a fairly even pressure level without any large fluctuations. Cf. Lundberg (13).)

**Case 3:** A. J. Born October 14, 1958. Born with a cervical meningocele and Arnold-Chiari malformation. October 14, 1958: excision of cervical meningocele. No complications in the immediate postoperative period, but in the following weeks he showed increasing signs of hydrocephalus with rapid increase in head circumference (Fig. 6). November 1958, lumbar encephalography: communicating hydrocephalus. December 12, 1958, operation: Spitz-Holter ventriculo-caval shunt. April 1959: increasing signs of septicemia, no signs of infection in the operative region; valve functioning well. Septicemia controlled with antibiotics. August 1959, pediatric examination: mental and motor development somewhat retarded. Lumbar encephalography: increase in brain substance, VFP measurement: normal.

**Case 4:** B. J. Born May 31, 1958. At birth meningocele in the lumbo-sacral region without neurologic symptoms. Operation June 1958: excision of the meningocele. After this operation rapidly increasing head circumference (Fig. 7). Encephalography August 1958: hydrocephalus. Encephalography January 1959 showed further dilatation of the ventricles. January 20, 1959, operation: Spitz-Holter ventriculo-caval shunt. No complications. August 1959, control examination: pediatric examination, normal development; lumbar encephalography showed increase in brain substance, 39 mm compared to 30 mm before operation (Fig. 8). Recording of the CSFP (lumbar needle) under pentothal anesthesia showed a mean pressure of about 2 mm Hg.

**Case 5:** M. F. Born September 11, 1958. Immediately after birth, rapid increase in

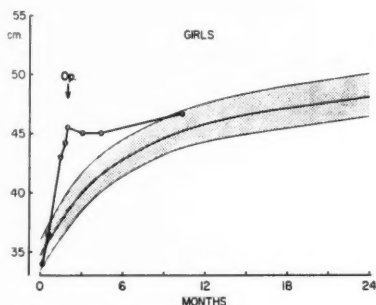


Fig. 6. Case 3. Ordinate: head circumference in cm. Abscissa: age in months.

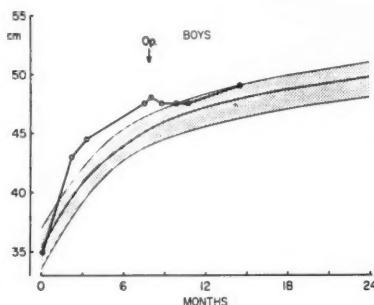
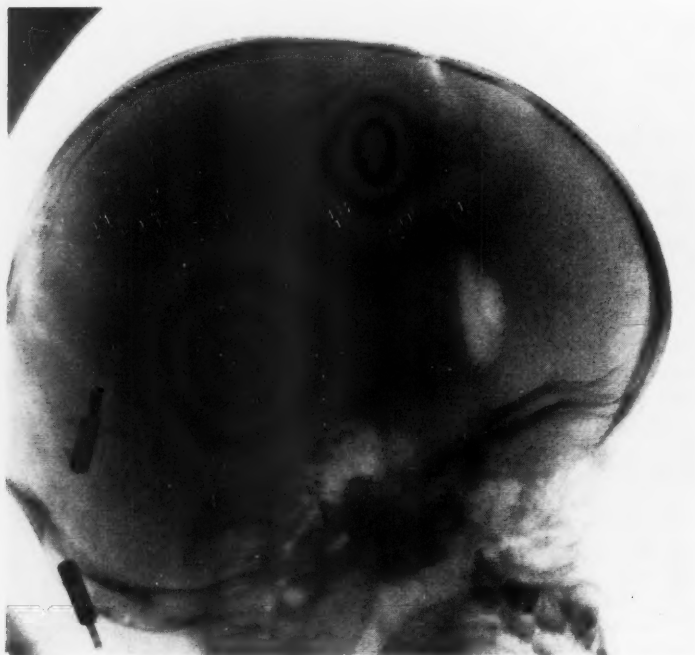


Fig. 7. Case 4. Ordinate: head circumference in cm. Abscissa: age in months.



a.



b.

Fig. 8. Case 4. a. preoperative, b. postoperative encephalography. See text.

head circumference of unknown etiology (Fig. 8). Encephalography March 1959: communicating hydrocephalus. Recording of the VFP: mean pressure about 12 mm Hg. April 27, 1959, operation: Spitz-Holter ventriculo-caval shunt. August 13, 1959, control examination: pediatric examination, mental development normal, motor development somewhat retarded; lumbar encephalography and VFP recording, increase in brain substance; mean VFP about 5 mm Hg. September 30, 1959: valve not functioning. Reoperation required, September 30, 1959: examination of shunt showed the right jugular vein to be obstructed. November 25, 1959: ventriculo-venous shunt on the left side. No postoperative complications. Valve functioning well.

### Comments

Among these five operated cases there were no postoperative deaths. Three reoperations were necessary. In one case the obstruction was localized to the jugular vein and was probably due to thrombosis. This failure was most probably caused by the catheter being too short. In one other patient two reoperations were necessary within a time of only two weeks, both due to obstruction which, by the pumping technique, was located to a site proximal to the valve. At the first reoperation no block of the ventricular catheter was found. At the second reoperation the passage through the ventricular cannula was found to be obstructed, probably because the tip was embedded in brain substance. In one case there were prolonged signs of sepsis (*Staphylococcus albus*), finally controlled by antibiotics. In this case there were no signs of infection in the operative field and no signs of thrombosis obstructing the caval catheter. At the end of the follow-up time (November 1959) all valves were functioning well.

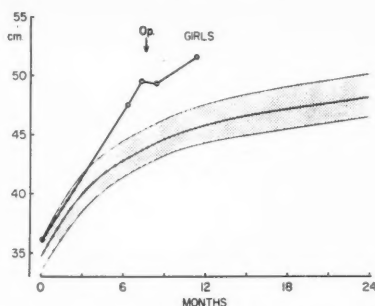


Fig. 9. Case 5. Ordinate: head circumference in cm. Abscissa: age in months.

The present valve was developed early in 1956 and has now been used in over five thousand cases all over the world (8). Spitz (23) has used the valve in over five hundred cases and he regards the results as very gratifying. At the beginning, reoperations were necessary in about thirty per cent but this figure has later been reduced to about ten per cent. Carrington (2) has recently presented a series of fifty patients where the Holter valve had been used. In forty-three, the valves were functioning well. Ten reoperations were necessary and eight of these were due to obstruction of the caval catheter (thrombosis).

One of the main arguments against this operation has been the factor of body growth. It has, however, been estimated that the distance from mastoid to xiphoid increases only 6 cm in the first years of life, and the new silicone tubing has the capacity of 300 per cent stretch.

In the treatment of hydrocephalus the fact must be considered that in about 40 per cent of the cases arrest will occur spontaneously (12). In such cases a shunt may be of benefit even if it should stop functioning after the acute symptoms have

subsided. The early diagnosis and treatment of infantile hydrocephalus is of great importance, in view of the rapid reduction of brain substance that may result from increased intraventricular pressure.

It is too early to evaluate fully the shunt operation with the Holter valve but the results so far are promising.

### Addendum

The Spitz-Holter procedure has also been used for palliative treatment of an adult patient with intracranial hypertension, the origin of which was not diagnosed until post mortem.

*Case 6: Diffuse melanosis of the meninges with intracranial hypertension.*

T. S. A., a man, aged 44, was admitted to the Neurosurgical Department in Lund because of signs of severe intracranial hypertension. His disease had started four months previously with headache, vomiting, confusion and unsteadiness of gait. Eventually he became more and more confused and agitated and he was repeatedly treated in a mental hospital. Three months after onset bilateral papilledema was found. Encephalography showed some herniation of the cerebellar tonsils and moderate widening of the ventricles, but no signs of a space-occupying lesion. There were no cells in the cerebrospinal fluid but the protein content was considerably increased. Vertebral angiography was normal.

Six months after onset he was admitted to the Neurological Department in Lund. He was then completely confused and disoriented, hallucinated, agitated and restless. Stiffness of the neck, paresis of the right abducens nerve and bilateral papilledema of 5 diopters was recorded. He was admitted to the Neurosurgical Department because of increasing signs of intracranial hypertension.

In addition to the above-mentioned signs, he had attacks of impaired conscious-

ness, increased agitation and stiffness of the neck and irregular respiration. Ventriculography showed the same state as at the previous encephalography, i.e. no signs of any localized lesion and free passage of the gas from the ventricles to the subarachnoidal space. The patient was treated by repeated lumbar punctures, which relieved his symptoms of intracranial hypertension. The protein content of the cerebrospinal fluid was high (1000–2000 mg per cent). Later, continuous drainage of the right lateral ventricle was instituted using a uni-directional valve. By this means the VFP was kept at a normal or slightly elevated level during a total of about four weeks. During this period the patient was less restless and agitated and there was no impairment of consciousness, but his mental state was otherwise unchanged. A slight regression of his papilledema was observed by the ophthalmologists. The valve was sometimes closed and the VFP recorded for a short period (Figs. 10a and 10b).

The shunt operation was performed 8 weeks after admission to the Neurosurgical Department. During the first days the pressure was low (Fig. 10c). Three days postoperatively the pressure started to rise but could still be temporarily reduced by pumping the valve (Fig. 11a). Eight days after operation when the ventricular cannula was removed the VFP was still above normal. At a control ventriculometry three weeks later the VFP was about 4 mm Hg and the same level was recorded 3½ months postoperatively (Figs. 11b and 11c). The valve functioned well as shown in Fig. 12. At this time the patient was still severely confused and disoriented. His general condition had deteriorated, he was permanently bedridden and severely emaciated. As compared with the preoperative state the papilledema had diminished but there was still a protrusion of 2 diopters in the left eye and 4 diopters in the right. Some vision was preserved but how much was impossible to assess. The protein content in the ventricular fluid was still high (about 200 mg per cent). The patient was transferred to his local

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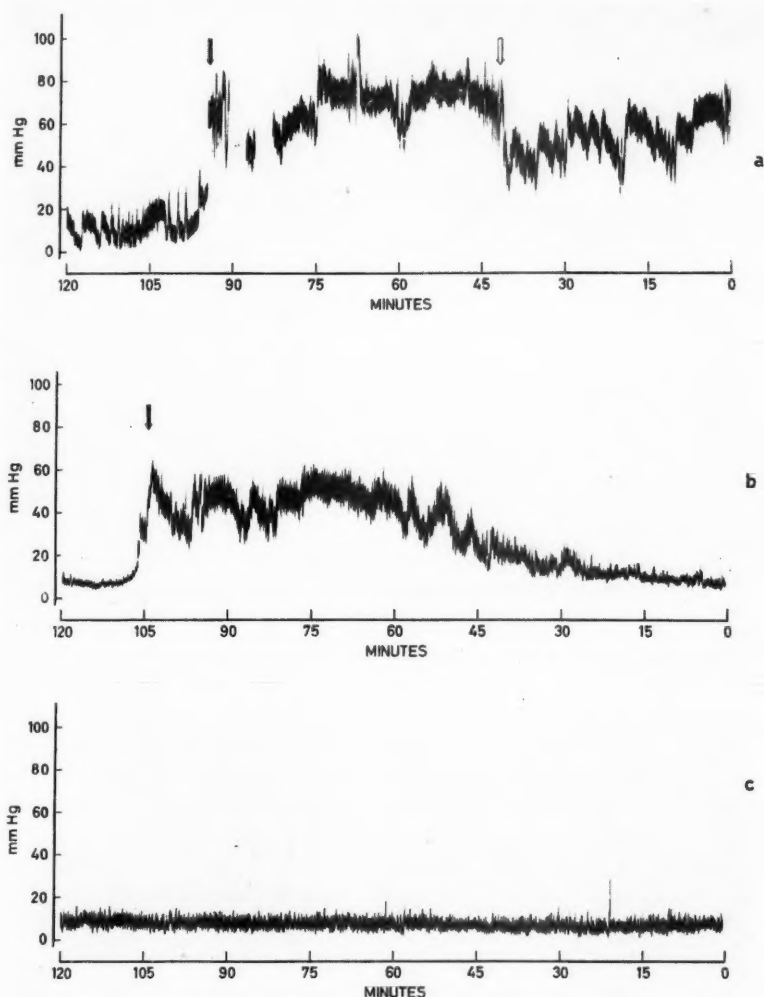


Fig. 10. Case 6. a and b. VFP before shunt operation. Unfilled arrow: Respiratory distress. Filled arrow: Tapping of ventricular fluid. c. VFP first day postop.—The curves should be read from right to left.

hospital where he died 2 months later, i.e. 6 months after the shunt operation. From the subsequent course of the disease it appeared that the valve was functioning until death.

#### Postmortem examination

Examination of the brain showed the meninges on the base of the brain to be the seat of a heavy neoplastic infiltration. There

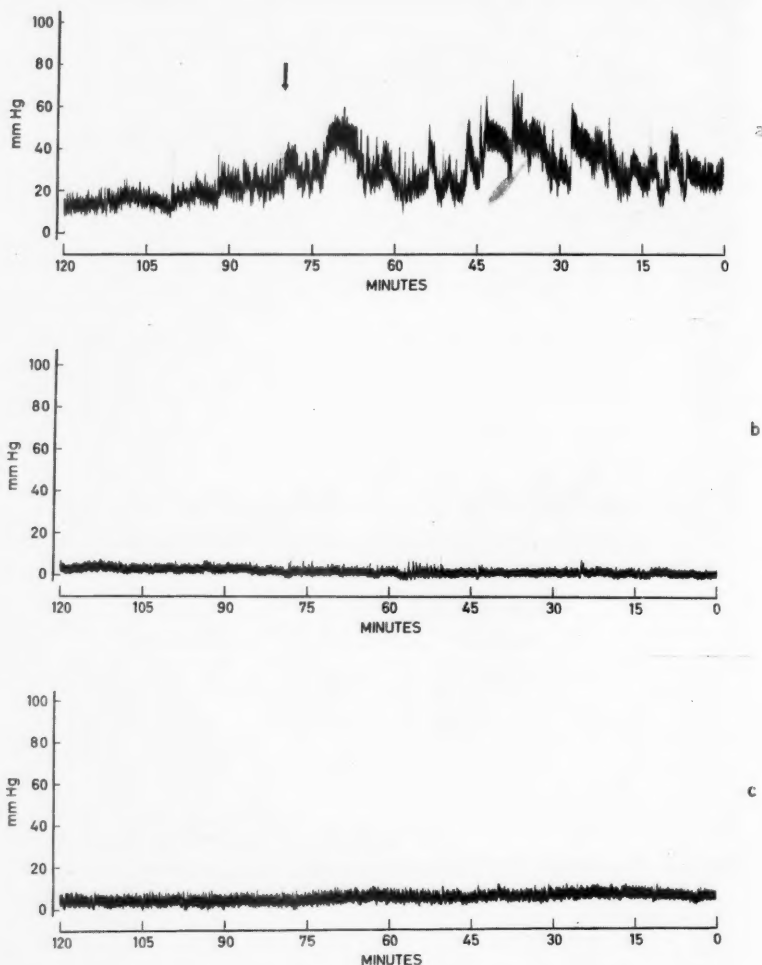


Fig. 11. Case 6. a. VFP 3rd day postop. Arrow indicates when valve is first compressed. b. VFP 3 weeks postop. c. VFP 3½ months postop. The curves should be read from right to left.

was moderate widening of the third and fourth ventricles but no appreciable widening and no disconfiguration of the lateral ventricles. Histological examination of the meninges at the base of the brain and on the floor of the third ventricle showed invasion of melanotic tumor cells. There were no signs

of inflammatory reaction or infection of the walls or the lateral ventricles or the meninges of the frontal lobes.

*Comments:* This case provided us with valuable experience. It showed that intracranial hypertension may be relieved

for a long time by the Spitz-Holter shunt, even if the ventricles are small and the fluid pathways patent. The procedure may thus be useful in selected cases of so called pseudotumor cerebri and replace subtemporal decompression, which has been widely used to relieve headache and preserve vision in such cases, but probably with limited benefit. The ventriculo-venous shunt may also be used as a palliative measure in selected patients with inoperable brain tumours and in patients where the intracranial hypertension is not relieved by excising a tumor, provided that shunting between the ventricles and the subarachnoidal space is not expected to be of benefit.

### Summary

A preliminary report is given of 5 cases of infantile hydrocephalus where ventriculo-venous shunt operations using the Spitz-Holter technique were performed. There were no postoperative deaths. In all cases pre- and postoperative measurements of head circumference, pneumography of the ventricular system, recordings of the ventricular fluid pressure and examinations by pediatricians with regard to mental and physical development were performed. The follow-up time ranged between 6 and 12 months. Three reoperations of the shunt were necessary in two patients, after which all valves

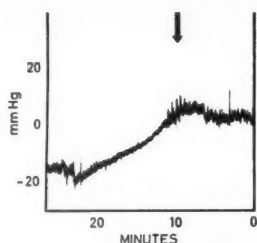


Fig. 12. Cases 6, VFP  $3\frac{1}{2}$  months postoperatively. Arrow indicates when valve is first compressed.

were functioning well. In all 5 cases interruption of the rapid increase in the head circumference occurred and a low ventricular fluid pressure was found postoperatively. In 4 cases postoperative pneumography suggested an increase in brain bulk. Normal mental development was found in 4 of the cases.

In addition a brief report is given of a case of increased intracranial pressure due to diffuse melanosis of the meninges in an adult. In this case a Spitz-Holter shunt operation reduced a preoperatively high and widely fluctuating ventricular fluid pressure to a low, even level during an observation time of 6 months. It is suggested that the Spitz-Holter operation may be of benefit in cases of severe intracranial hypertension where removal of an intracranial tumour or an intracranial shunt operation does not give relief.

### References

1. ANDERSSON, H., and BOHM, E.: Om infantil hydrocephalus och dess behandling. *Svensk Läkartidn*, 44: 3081, 1958.
2. CARRINGTON, K. W.: Ventriculo-venous shunt using the Holter valve as a treatment of hydrocephalus. *J Mich Med Soc*, 58: 373-376, 383, 1959.
3. CONE, W. V., LEWIS, R. D., and JACKSON, I. J.: Pres. at meeting of *Amer Coll Phys*, Montreal, Canada, Sept., 23, 1949.
4. DANDY, W. E.: Experimental hydrocephalus. *Ann Surg*, 70: 129, 1919.
5. DAVIDOFF, L.: Treatment of hydrocephalus. *Arch Surg*, 18: 1737, 1929.



6. GÄRTNER: Cited by Kausch.
7. HAKIM, S., JIMENEZ, A., and ROSAS, F.: Drainage of the cerebrospinal fluid into the spinal epidural space: a new technique for the treatment of hydrocephalus. *Acta Neurochir (Wien)*, 4:3, 1955.
8. HOLTER, J. W.: Personal communication. 1959.
9. HYNDMAN, O. R.: Hydrocephalus. *J Neurosurg*, 3:426-443, 1946.
10. INGRAHAM, F. D., MATSON, D. D., ALEXANDER JR, E., and WOODS, R. P.: Studies in the treatment of experimental hydrocephalus. *J Neuropath Exp Neurol*, 7:123-143, 1948.
11. KAUSCH, W.: Die Behandlung des Hydrocephalus der kleinen Kinder. *Arch Klin Chir*, 87:709-796, 1908.
12. LAURENCE, K. M.: Communication at 29th Annual Meeting of Brit Paediat Ass, 1958.
13. LUNDBERG, N. G.: Continuous recording and control of ventricular fluid pressure in neurosurgical practice. *Acta Psychiat Scand, Suppl 149*, Copenhagen, 1960.
14. MATSON, D. D.: A new operation for the treatment of communicating hydrocephalus. Report of a case secondary to generalized meningitis. *J Neurosurg*, 6:238-247, 1949.
15. MATSON, D. D.: Ventriculo-ureterostomy. *J Neurosurg*, 8:398-404, 1951.
16. MACNAB, G. H.: Hydrocephalus of infancy. Recent Advances in Paediatrics. Ed. by Douglas Gairdner, J. & A. Churchill, Ltd., London, 1958.
17. NULSEN, F. E., and SPITZ, E. B.: Treatment of hydrocephalus by direct shunt from ventricle to jugular vein. *Surgical Forum, Amer Coll Surg, Philadelphia*, 2:399-403, 1952.
18. PAYR, E.: Drainage der Hirnventrikel mittelst frei transplantierter Blutgefäße. Bemerkungen über Hydrocephalus. *Arch Klin Chir*, 87:801-885, 1908.
19. PAYR, E.: Über Ventrikeldrainage bei Hydrocephalus. *Verh Deutsch Ges Chir*, 40:515-535, 1911.
20. PUDENZ, R. H., RUSSEL, F. E., HURD, A. H., and SHELDEN, C. H.: Ventriculo-auriculostomy. Technique for shunting cerebrospinal fluid into the right auricle. *J Neurosurg*, 14:171-179, 1957.
21. SCARFF, J. E.: Nonobstructive hydrocephalus. *J Neurosurg*, 9:164, 1952.
22. SPITZ, E., and KOOP, C.: Forum meeting. *Amer Coll Surg*, Sept. 1952.
23. SPITZ, E.: Personal communication, 1959, 1960.
24. STUART, H. C., and STEVENSON, S. S.: Physical growth and development. In Nelson: "Textbook of Pediatrics", Philadelphia & London, 1954.

Submitted Dec. 10, 1960

Accepted Dec. 19, 1960

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## PROGRESS IN PEDIATRICS

### Symposium on Haemolytic Disorders in Childhood

From the Departments of Paediatrics and Clinical Chemistry, University Hospital, Uppsala, Sweden

#### Contents

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#### Bo Vahlquist:<sup>1</sup> Introduction

During the last week of September, 1960, the Swedish Paediatric Society had the pleasure of the company of 20 members of the British Paediatric Association as its guests. The group visited Gothenburg, Stockholm, and Uppsala. Meetings were arranged at each of these university clinics, the guests reading papers and the hosts arranging symposia. The subject of the Uppsala symposium was "Haemolytic Disorders in Childhood", and the choice was influenced by the fact that this subject has for many years been one of the main research topics of the Department of Paediatrics in Uppsala.

There is probably no field within haematology which has developed so rapidly in the last few decades as that relating to the haemolytic disorders. As a matter of fact, so many new laboratory methods have been

introduced, and so many new observations made, that it is becoming increasingly difficult for the individual physician to keep abreast with them all. The papers now published do not embrace the iso-immunization disorders, a field large enough to deserve a symposium of its own. Even with this limitation, the subject to be covered is so vast that many parts of it can be touched upon only very briefly. Attention is directed chiefly to disease states of particular topical interest. You will note that many of the disease mechanisms discussed are so similar in both children and adults that a sharp demarcation between the age groups would be artificial.

Haemolytic mechanisms are at work in childhood considerably more often than is usually recognized. Furthermore the application of the complete armamentarium of serological and chemical laboratory methods of to-day leave an ever-diminishing group of cases which are not clarified with respect to mechanism and severity of disease.

#### Stig Sjölin:<sup>2</sup> Clinical picture, classification, and diagnosis

The clinical picture in haemolytic states varies greatly. On the whole there is a close relationship between the clinical picture and the degree of haemolysis, pronounced haemolysis giving pronounced clinical signs. On the other hand, the clinical picture is fundamentally independent of such factors as the cause of the haemolysis and the patient's age. The only striking and important exceptions to this rule are seen during the first months of life [27].

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In marked haemolysis the presenting signs are pallor, lassitude, restlessness, jaundice, pains in the back and extremities, pyrexia, palpitations, and dyspnoea. The clinical picture and preliminary examination alone will usually suffice to give the diagnosis in such cases. There is always anaemia, and as a rule hyperbilirubinaemia, marked reticulocytosis, and anisocytosis. Normoblasts are often found in the peripheral blood. There is also commonly leukocytosis and a "shift to the left". Haemolysis may be so rapid that haemoglobinaemia and haemoglobinuria result. The serum haptoglobin is practically always nil. There is always increased excretion of urobilinogen in urine and faeces. Splenomegaly is common, and enlargement of the liver may occur as well.

Slight hyperhaemolysis, however, is most commonly characterized by the absence of physical signs. There is sometimes slight jaundice, with no anaemia and no complaints on the patient's part. In more chronic states, for example, some cases of congenital spherocytosis, the patient may remain symptom-free for long periods. In such cases attention is often drawn to the illness only during acute exacerbations or through attacks of abdominal pain or symptoms of gall-bladder disease. The diagnosis in such cases may be very elusive, and may be entirely dependent on the physician's powers of observation. Particular vigilance is called for when there is no anaemia. The degree of hyperhaemolysis is then so modest that the bone-marrow, by increasing its production, is able to compensate for the loss due to haemolysis. Such cases of compensated haemolytic disease may be associated with hyperbilirubinaemia, at any rate at times, and the haptoglobin level may be low, but slight reticulocytosis is commonly the only sign. The reticulocytosis may never be regarded as more than an indirect sign of hyperhaemolysis, however, and it is never permissible to base the diagnosis of a haemolytic disorder solely on the finding of an increased reticulocyte count. It is in the case of these patients in particular, who are

relatively free from symptoms, that the clinician requires the help of the special laboratory to determine the red-cell survival and thus to arrive at the true diagnosis.

When the investigation of a case has proceeded so far that hyperhaemolysis has been demonstrated, there remains the important task of establishing exactly what type of haemolytic disorder the patient is suffering from. Even though our knowledge of the causes of the haemolytic states is still incomplete, the clearest and most useful classification from the point of view of the clinician is that based on pathogenesis. In general these states may be divided into 3 groups (Table 1). The first includes those disorders in which the basic fault is a defect in the red cells. In the second group we have the cases in which normal red cells are produced and the haemolytic process is caused by some extracorporeal factor. Finally, the third group includes cases in which it is known that haemolysis is due to a combination of intra- and extracorporeal factors.

The chances of distinguishing these three groups are as a rule fairly good, but may be difficult if the patient has recently received a blood transfusion. If possible, blood should not be administered until the diagnosis is clear.

In this paper I am only going to discuss some of the most important of these haemolytic states and only those factors and laboratory tests that are not coming up later in this Symposium.

### *Intracorporeal defects*

Most of the haemolytic anaemias due to intracorporeal factors are hereditary. In these states a good deal of help is, of course, to be had from the family history and investigation of the patient's relations, but it must be remembered that there are patients with hereditary haemolytic anaemia whose parents and siblings are healthy. Hereditary haemolytic anaemia may produce symptoms at birth. In this connexion it must be stressed that the red cells of the newborn are macrocytic and contain foetal haemoglobin,

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TABLE 1. *Classification of haemolytic disorders.**Intracorpuseular defects*

- Hereditary spherocytosis
- Hereditary elliptocytosis
- Hereditary non-spherocytic haemolytic anaemias
- Congenital Heinz-body anaemia
- Congenital haemolytic anaemia associated with porphyria
- Sporadic congenital spherocytosis associated with congenital hypoplastic thrombocytopenia and malformations
- Infantile pyknotocytosis?
- Thalassaemia
- Haemoglobinopathies
- Paroxysmal nocturnal haemoglobinuria

*Extracorpuseular causes*

- Haemolytic anaemias caused by isoagglutinins
  - Haemolytic disease of the newborn
  - Mismatched transfusions (anti-A, anti-B, anti-Rh, etc.)
- Idiopathic haemolytic anaemias
  - Antibodies demonstrable (auto-immune haemolytic anaemias)
  - No antibodies found
- Symptomatic haemolytic anaemias
  - Leukaemia, malignant lymphomas, osteopetrosis, disseminated lupus erythematosus, thrombotic thrombocytopenic purpura, cirrhosis of the liver
- Haemolytic anaemias caused by infections
  - Malaria, virus pneumonia, infectious mononucleosis
- Haemolytic anaemias caused by chemical agents
  - Phenylhydrazine, naphthalene, aniline, lead, sulphanilamide, vitamin K, etc.

*Combination of intra- and extracorpuseular factors*

- Chemical agents: primaquine, naphthalene, sulphanilamide (vitamin K?)
- Vegetable agents: fava bean

and that their enzyme pattern is different from that of red cells of adults [97], and their osmotic resistance is higher [80]. The characteristic blood picture of several hereditary haemolytic syndromes is therefore often modified in the neonatal period and during early infancy [10, 27].

*Hereditary spherocytosis* is inherited as a Mendelian dominant, and is most frequently recognized for the first time during childhood or early adulthood, but cases have been diagnosed at or within a few days of birth [56, 73]. The chief feature characterizing the red cells in this condition is microspherocytosis. And providing the technique is careful, the osmotic resistance can usually be shown to be decreased. The chances of demonstrating the anomaly of the red cells are improved if the cells are first incubated at 37°C for 2-4 hours [19]. The osmotic resistance is then usually found to be greatly lowered. The degree of spontaneous haemolysis is in-

creased after incubation; and if glucose is added autohaemolysis is diminished by the same degree as in normal red cells [19]. Aplastic crises are seen in hereditary spherocytosis. These cases are characterized by a sudden cessation of bone marrow function with varying degrees of anaemia, leukopenia, and thrombocytopenia. From the rather extensive studies that have been carried out it now seems likely that hyperhaemolysis in hereditary spherocytosis is due to a genetically determined defect in the metabolism of the red cells [69, 70].

*Non-spherocytic haemolytic anaemia* also seems to be inherited as a Mendelian dominant. This disease, which is relatively rare, has been described in newborn infants, and may resemble severe erythroblastosis foetalis [53]. In most of the cases that have been published, findings such as jaundice and anaemia were present from early infancy. There are no microspherocytes, and

the osmotic resistance is normal. Selwyn & Dacie have shown that there are at least two main types of non-spherocytic anaemias [76]. Type I is characterized by a moderate degree of ovalocytosis; there is a tendency to abnormally increased osmotic resistance after incubation; the autohaemolysis rate is within the normal range or only slightly increased; and the addition of glucose reduces the degree of autohaemolysis, but less so than with normal cells. Type II cases have rounded macrocytes; after incubation the osmotic resistance of the red cells decreases markedly; the rate of autohaemolysis is strikingly increased; and the addition of glucose increases rather than decreases autohaemolysis. Marked basophilic stippling is sometimes found, and it is possible that these cases form a third group [19]. The essential defect in the red cells of hereditary non-spherocytic haemolytic anaemia seems to be biochemical and to differ in nature from that in hereditary spherocytosis [77, 98].

*Congenital haemolytic anaemia with marked Heinz-body formation* is a rare disease, predominantly affecting premature infants and probably caused by a congenital defect of the red cells [4, 19]. As a rule the disorder is transient (although sometimes fatal), but some cases seem to persist into late childhood. This group includes only spontaneous cases, that is, those cases where no toxic agents such as aniline, sulphanilamide, or vitamin K can be incriminated.

*Infantile pyknocytosis* is a "new" disease quite recently described by Tuffy, Brown & Zuelzer [89]. They published the cases of 11 newborn infants, both premature and full-term, with acute severe haemolytic anaemia characterized by an increased number of pyknocytes or burr cells. These cells are distorted and contracted red cells with spiny projections, and seem to occur regularly in small numbers in infants up to the age of 2-3 months. The disease is associated with neonatal hyperbilirubinaemia. It is transient, and responds to transfusion therapy. Genetic factors probably play a part in its development.

*Thalassaemia minor* is often difficult to

diagnose. It represents the heterozygous state of a partially dominant autosomal gene, and is characterized by the presence of numerous target cells, raised osmotic resistance, and increased concentration of haemoglobin A<sub>2</sub>. In many cases small quantities of foetal haemoglobin can also be demonstrated. The rate of destruction of red cells is usually normal. *Thalassaemia major*, or Cooley's anaemia, represents the homozygous condition. The illness usually makes its first appearance during infancy. The most typical features are marked haemolytic anaemia with pale, irregular, sometimes fragmented red cells and target cells, increased osmotic resistance, and a high concentration of foetal haemoglobin. No abnormal haemoglobin is found. *Thalassaemia* is therefore not to be considered a true haemoglobinopathy. It is not confined solely to persons of Mediterranean ancestry.

*Paroxysmal nocturnal haemoglobinuria* is a disease of adults, and very few cases have been described in children [18]. There is never a family history of the condition, in spite of the disease being due to an intracorpuseular defect.

### *Extracorpuseular causes*

Haemolytic anaemias due to extracorpuseular factors are usually classified as acquired haemolytic anaemias. A large number of these anaemias are due to antibodies. Our most important diagnostic aid in these cases is Coombs's direct test.

*Haemolytic disease of the newborn* is characterized by the fact that the antibodies are transferred passively. The haemolytic process is consequently transient, the degree of haemolysis subsiding parallel to the natural decay of the immune globulins.

*Idiopathic haemolytic anaemias*. Among the haemolytic anaemias there are always cases in which no aetiological factor can be discovered. These are classed as idiopathic haemolytic anaemias. The clinical picture varies widely, but the signs are rarely as mild as those sometimes encountered in hereditary spherocytosis. In children the

onset is often abrupt. Cases with an acute onset, short duration, and spontaneous recovery have often been referred to as Lederer's anaemia. In most cases Coombs's direct reaction is positive, and "warm" antibodies can usually be demonstrated. In these cases the disorder is called auto-immune haemolytic anaemia. The fact that antibodies cannot be demonstrated in certain cases may be due to imperfections in the serological technique. Idiopathic haemolytic anaemias can be accompanied by thrombocytopenia. Aplastic crises are rare. Idiopathic haemolytic anaemias occur rarely during the first months of life, probably because the capacity for forming antibodies is then slight. The youngest patients described were 2½, 5, and 5½ months old [18, 55].

*Symptomatic haemolytic anaemia.* Since reliable methods have become available for the measurement of the life-span of the red cells it has been found that the usually slight, but sometimes grave, anaemia that accompanies many prolonged, serious disease states is commonly, at least in part, due to shortened red-cell survival. Sometimes cases of this type may show marked haemolytic signs. In several cases of symptomatic haemolytic anaemia Coombs's direct test has been positive, and it is probable that the mechanism of haemolysis is similar to that in the auto-immune haemolytic disorders. A number of the states that may cause symptomatic haemolytic anaemias occur during childhood, for example, leukaemia, thrombotic thrombocytopenic purpura, osteopetrosis, lupus erythematosus, and cirrhosis of the liver [12, 18, 82, 88, 92, 93].

*Haemolytic anaemias due to virus infections* are rare in childhood. Virus pneumonia and infectious mononucleosis seem to be the commonest causes, but cases have also been described following measles and herpes simplex [18, 35, 87]. Antibodies have been incriminated, and in cases following virus pneumonia they have been of the "cold" variety.

*Haemolytic anaemias caused by chemical agents.* Among chemical agents that may induce haemolytic anaemia synthetic water-

soluble vitamin K is of special interest to the paediatrician [4, 96, 97]. It is now certain that excessive doses of vitamin K given to new-born premature infants may result in progressive haemolytic anaemia during the first weeks of life. It is uncertain, however, whether this is true of all premature infants or only of those with sensitive cells (see below). The blood picture is characterized by large numbers of Heinz inclusion bodies and numerous distorted and fragmented red cells. The disease is self-limiting, but death from kernicterus has been reported.

#### *Combination of intra- and extracorporeal factors*

Concerning the third main group, combined intra- and extracorporeal factors, I should first like to point out that they provide examples of a "new" mechanism of haemolysis. It has for long been known that certain persons, chiefly negroes, develop haemolytic anaemia after receiving the antimalarial drug primaquine, and that others, mostly of Italian and Greek descent, develop haemolytic anaemia after eating fava beans or after inhaling fava-bean pollen [9]. It has since been shown that the red cells of these persons are sensitive to the respective agents, exposure leading to haemolysis. Owing to an inherent biochemical abnormality, these primaquine-sensitive cells form Heinz bodies more readily than non-sensitive cells. It is highly probable that these findings will also prove significant in the elucidation of other haemolytic anaemias caused by chemical agents in sensitive persons. There is, for example, much to suggest that some vitamin K-, naphthalene-, and sulphanilamide-induced haemolytic anaemias belong to this group [94].

*The importance of red-cell survival studies.* It is clear that with the aid of fairly simple laboratory tests we are often able to establish with certainty the nature of a given patient's haemolytic anaemia. On the other hand, it must be stressed that advanced laboratory facilities are frequently required in order to arrive at the correct diagnosis. This



TABLE 2. *Summary of the investigation of a patient with suspected haemolytic anaemia.*  
(Modified after DE GRUCHY, 1958.)

### *History*

- Age at onset of symptoms
- Race
- Jaundice
- Colour of urine and faeces
- Crises
- Cholelithiasis
- Family history
- Symptoms suggestive of disorders causing symptomatic acquired haemolytic anaemias
- Drugs

### *Physical examination*

- Jaundice, splenomegaly, hepatomegaly
- General development, height, facies, presence of congenital abnormalities, leg ulceration or pigmentation (hereditary haemolytic anaemias)
- Signs of disorders causing symptomatic acquired haemolytic anaemia, especially lymph-node enlargement and purpura

### *Special investigations*

#### *A. Essential investigations for all cases*

- Full blood examination, with special reference to:
  - Morphology of red cells in a well-made and well-stained blood film (note especially spherocytosis, auto-agglutination, fragmentation, inclusion bodies)
  - Reticulocyte count
  - Serum bilirubin estimation
  - Osmotic resistance
  - Direct Coombs's test
  - Examination of urine for urobilinogen, haemoglobin, haemosiderin

#### *B. Further investigations required in some cases*

- Estimation of faecal urobilinogen
- Measurement of red-cell life span with Cr<sup>51</sup> (or DFP<sup>32</sup>) combined with surface counting over liver and spleen
- Measurement of red-cell destruction with carbon monoxide
- Autohaemolysis after incubation, with and without added glucose (hereditary spherocytic and non-spherocytic haemolytic anaemias)
- Osmotic resistance after incubation (hereditary spherocytosis)
- Examination of plasma for haemoglobin and methaemalbumin
- Estimation of haptoglobin concentration
- X-ray of skull, hands, and long bones (hereditary haemolytic anaemias)
- Sickle-cell test (sickle-cell anaemias)
- Tests for abnormal haemoglobin types (hereditary haemolytic anaemias)
  - (a) Paper, starch block, or agar gel electrophoresis
  - (b) Chromatography
  - (c) Alkali denaturation technique
- Investigation of relatives (hereditary haemolytic anaemias)
- Examination of cells for methaemoglobin and sulphaemoglobin (chemical haemolytic anaemia)
- Heinz-body preparations (chemical haemolytic anaemia)
- Serum cold agglutinins and warm agglutinins (acquired haemolytic anaemias)
- Investigations to demonstrate aetiology in symptomatic acquired haemolytic anaemia, especially LE-cell test and lymph-node biopsy
- Wassermann test (paroxysmal cold haemoglobinuria: N.B. false positives in acquired haemolytic anaemias)
- Donath-Landsteiner test (paroxysmal cold haemoglobinuria)
- Ham's acidified-serum test (paroxysmal nocturnal haemoglobinuria)



will be further illustrated at a later stage of the Symposium. In the present connexion I wish only to emphasize the value of repeated red-cell survival studies, especially in cases in which there is doubt concerning the main group to which the case should be assigned. I think this is best illustrated by an example. In a case of osteopetrosis that we examined we were able to demonstrate with the aid of radioactive chromium that the red cells of the patient had a reduced survival time following autotransfusion [82]. In other words, haemolytic anaemia was present. Chromium-tagged red cells from a healthy donor behaved in the same manner when injected into the patient. These investigations thus established the fact that an extracorporeal factor was at work, but it was not until the patient's chromium-tagged red cells had proved to survive normally in a healthy recipient that it became clear that hyperhaemolysis was due exclusively to an extracorporeal factor. Similarly, it is possible to demonstrate intracorporeal defects, and even combined intra- and extracorporeal factors. And survival studies on red cells of varying genotype are of great value in investigating the part played by different antibodies in causing haemolysis in individual cases [45].

When a haemolytic anaemia is suspected, a careful clinical and laboratory investigation is always needed in order to establish the correct diagnosis. In analysing such cases the procedures listed in Table 2 may serve as a guide.

#### *Claes Högman*:<sup>1</sup> Serological aspects of pathogenesis and diagnosis

It is now generally accepted that acquired haemolytic anaemia (AHA) can be due to immunological mechanisms. Antibodies, either produced by another individual (iso-antibodies) or by the patient himself (auto-antibodies) react with antigens of the patient's erythrocytes, resulting in shortening

of the life-span of the cells. "Haemolytic disease of the newborn", as a rule due to passively transferred Rh or ABO antibodies, is the commonest immune haemolytic anaemia of childhood in our part of the world. This disorder will not be discussed at this Symposium, however.

In the other type of AHA, that due to auto-antibodies, serological methods such as the antiglobulin reaction (Coombs's test) are positive, indicating the presence of antibody-like globulins on the patient's erythrocytes. It is not known, however, whether the globulins coating the red cells are true auto-antibodies, or whether they are antibody-like pathological proteins. From a clinical point of view they may be regarded as true antibodies. They can be classified according to the temperature of their optimum reaction *in vitro* [18]. The warm antibody has its optimum at 37°C, whereas the cold reacts best at temperatures just above zero, with some activity up to 28°–32°C but seldom at 37°C. Certain other properties of the antibodies are correlated to the optimum temperature, and are summarized in Table 3 cited from Dacie [20].

The anaemia caused by antibodies of the cold type is the rarest, and seems chiefly to affect patients above 40 years of age [22]. The warm type anaemia may appear equally frequently in children as in adults. The antibodies of the latter type are incomplete, that is, they are detectable by the antiglobulin technique but do not cause agglutination of red cells suspended in saline medium. The incomplete antibodies may, however, agglutinate red cells suspended in colloid-rich medium such as albumin, gum acacia, dextran, or polyvinylpyrrolidone (PVP). Sometimes the most sensitive reaction is obtained if the red cells are treated with certain proteolytic enzymes such as trypsin, papain, or ficin [60]. Red cells coated with incomplete auto-antibodies are usually not haemolysed in the presence of complement, as is often the case with the cold type antibodies [18, 19]. An exception from this rule is described by Dausset & Colombani [22]. In a series of 128 cases of AHA, four patients

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TABLE 3. *Characteristics of the auto-antibodies of acquired haemolytic anaemia (After DACIE, 1959.)*

	"Warm" type of antibody	"Cold" type of antibody
Nature		
Electrophoretic mobility	$\gamma$ -Globulin	$\gamma$ -Globulins (? $\beta$ -Globulins)
Sedimentation constant	S <sub>20,w</sub> 7	S <sub>20,w</sub> 16-19
Class of antibody		
Complete (agglutinating) or incomplete (non-agglutinating)	Usually incomplete	Complete
Haemolytic potentiality	Fails to haemolyse normal or trypsinized red cells or PNH red cells	Haemolyses normal red cells (sometimes only at pH 6.5-7.5), trypsinized red cells and PNH red cells
Antiglobulin reaction		
Presence of prozone	Frequent	No prozone
Effect of pH of patient's serum	Little effect	Agglutination usually increased at pH 6.5-7.5
Effect of heating patient's serum to 56° C	Little effect	Complete inhibition of agglutination
Effect of adding $\gamma$ -globulin to anti-globulin serum	Inhibition by small amounts of $\gamma$ -globulin	No inhibition by small amounts of $\gamma$ -globulin
Thermal range of antibody	Maximal at approximately 37° C	Not active at 37° C; just active at 28-32° C; activity greatly increased by cooling below 28° C
Specificity of antibody	"Non-specific", usually; anti-Rh plus "non-specific", occasionally; anti-Rh alone or other specificities, rare	"Non-specific" Anti-I

had antibodies of the warm type which, against the rule, acted as haemolysins. Three of these cases were children, 8 months to 2½ years of age, and they all showed a similar clinical picture, with pyrexia, haemoglobinuria, jaundice, and enlargement of the liver and spleen. The illness appeared after an upper respiratory infection, and rapid recovery occurred after transfusion and corticosteroid therapy.

The auto-antibodies may be found both in serum and on the patient's red cells or on the red cells only. They can be removed from the erythrocytes by elution, and subjected to analysis [18, 51]. The cold auto-antibodies always appear non-specific in routine testing, i.e. they react with all samples of red cells independent of their blood groups. In contrast, the warm antibodies sometimes

show a distinct serological specificity [49, 91], often within the Rh-system. Holländer & Batschelet [42] pointed out that the incidence of the different Rh-specific auto-antibodies is closely correlated to the incidence of the Rh-antigens in the population. Consequently, as the e-antigen is present in about 98% of all individuals, the anti-e would be expected to be one of the commonest specific auto-antibodies; and this, in fact, is the case. Dacie [20] suggests that the apparently non-specific antibodies (which are the commonest of the auto-antibodies) may also turn out to be specific. They could, in theory, be directed against a "public" antigen common to the vast majority of human individuals.<sup>1</sup>

<sup>1</sup> Support for this hypothesis has been given by Wiener, Unger, Cohen and Feldman (*Ann.*

In certain cases this specified diagnosis of the antibodies may not only be of theoretical interest but also of clinical value. It has been demonstrated [45, 61] in cases of AHA due to auto-anti-e that the patient's own red cells, which contained the e-antigen, were destroyed much more rapidly than transfused red cells lacking the e-antigen.

The clinical findings may indicate AHA, even though the serological reactions are all negative. The question of whether the disease is due to a non-immunological mechanism in these cases or whether the methods available are inadequate cannot be answered at present. It is to be remembered, however, that the reagent used in the antiglobulin test, which is considered to be the most reliable method available, is produced by animals, usually rabbits, and that the capacity of individual rabbits to form these antibodies varies greatly. The antiglobulin sera must be carefully selected, absorbed and tested, and should be used in at least two different dilutions [25, 60]. Furthermore, it is possible that in specific cases the patient's auto-antibodies may consist of pathological globulins not normally present, and that normal human serum is therefore not capable of stimulating the animals to produce the relevant anti-human globulins. By immunizing rabbits with the serum of a patient showing clinical signs of haemolytic anaemia, Evans & Weiser [28] obtained an antiglobulin serum which gave a positive antiglobulin reaction with the red cells of the patient in question, although other antiglobulin sera failed.

Addition of suitable amounts of human gamma-globulin to antiglobulin sera inhibits the anti-gammaglobulin, but not anti-

Intern. Med. 44, 221, 1956), who showed that 5 out of 22000 bloods reacted only very weakly with potent cold agglutinins. They concluded that an antigen called I was lacking in the red cells of these 5 individuals. This antigen is poorly developed at birth and increases in strength continuously during the 2nd to 12th month of life (Marsh: Brit. J. Haematol. 7, 200, 1961). The finding of Wiener *et al.* that the cold agglutinins show an anti-I specificity has been confirmed by Jenkins, Marsh, Noades, Tippet, Sanger and Race Vox Sang. 5, 97, 1960).

bodies against other globulins. Sera partially neutralized in this way react with red cells sensitized by certain types of human antibodies. This is true both with iso- and auto-antibodies [60]. Human auto-antibodies can thus be divided into a gamma-globulin and a non-gamma-globulin type. Recently it has been demonstrated [73] that the "non-gamma-globulin" reaction is due to rabbit anti-human globulin which combines not with the antibody itself but with human complement participating in the reaction.

The fixation of antibodies on the surface of erythrocytes apparently causes damage to the cells, reducing their life-span. The survival time of red cells has been studied by differential agglutination (Ashby technique) and by tagging red cells with radioactive isotopes, in recent years especially Cr<sup>51</sup> [60, 61]. Both quantitative and qualitative factors influence the rate at which the antibodies bring about destruction of the cells. Rh-positive red cells coated with large amounts of Rh (D) antibodies survive for a far shorter time than cells coated with a smaller amount of antibodies [48]. In the acute phase of haemolytic anaemia antibodies are often found in the serum as well as on the surface of the red cells, whereas no free antibodies may be found during remissions [18]. This indicates that excess antibody is formed in the acute phase. The presence of free antibodies in the plasma has been found to indicate a poor prognosis [22].

In some cases the red cells can be shown to undergo intravascular lysis, apparently by the action of serum complement; in other cases they are destroyed with the cooperation of such organs as the liver and the spleen [44, 45, 48, 49, 50, 62]. In the latter event little or no free haemoglobin is to be found in the plasma. By measuring the radioactivity over different areas of the body it is possible to get a rough idea of the accumulation in different organs. This in turn indicates that the organ in question is involved in the process of red-cell sequestration. Erythrocytes coated with incomplete antibodies are generally trapped in the spleen, whereas complete antibodies cause seques-

TABLE 4.

Subcellular parts	Main function	Presence in the mature erythrocyte
Nucleus	Source of genetic information (from DNA)	—
Mitochondria	Production of energy (ATP) through oxidation	—
Microsomes	Synthesis of proteins (enzymes)	—
Supernatant fraction	Glycolysis to (pyruvic acid and) lactic acid	+
	Purine and pyrimidine metabolism	(+)

tration in the liver [44, 48, 49, 50]. The site of destruction of the erythrocytes is also influenced by quantitative factors; the same serum may, in small doses, cause red-cell sequestration in the spleen, in bigger doses also in the liver [50]. Increased destruction of red cells probably due to an immunological mechanism is sometimes observed, although none of the serological reactions is positive [2, 81, 85].

As previously mentioned different co-factors are often necessary if an antigen-antibody reaction is to be apparent *in vitro*. This may well also be the case *in vivo*. For example, deficiency of complement may lower the effect of complement-fixing antibodies. The function of agglutinating factors present in pathological [38] or normal [43] sera is unknown. It is possible that such factors may increase the damaging effect of sensitization. Jandl *et al.* [48] found a higher rate of destruction of red cells from a patient with acquired haemolytic anaemia when the cells were injected into a patient with multiple myeloma than when they were injected into a healthy individual. The authors concluded that the hyperglobulinaemia was of importance. The occurrence of the agglutination *in vitro* of red cells coated with incomplete antibodies which takes place with the aid of certain large molecules would thus perhaps have a parallel *in vivo*.

#### Carl-Henric de Verdier:<sup>1</sup> Biochemical aspects of pathogenesis and diagnosis

The evolution of the erythroblast to a mature red blood corpuscle is an example of

development to a highly specialized cell. The morphological characteristics are the disappearance of nucleus, mitochondria, and microsomes (Table 4). These different subcellular particles have different biochemical functions which will disappear at the same time, and this means that the mature erythrocyte will have a very restricted metabolic pattern. In an ordinary cell, most high-energy compounds such as adenosine triphosphate (ATP) are formed by oxidative phosphorylation when substrates such as pyruvate are degraded through Krebs' citric-acid cycle in the mitochondria, and they are only to a smaller extent formed through glycolysis. In the erythrocyte ATP is formed only by glycolysis. When the haemoglobin synthesis is complete, the mitochondria will disappear, and the cell can no longer synthesize proteins, which means that it cannot replace consumed enzymes. This is important to bear in mind when discussing the pathogenesis of haemolytic anaemia.

There are several different pathways of intracellular metabolism, related to the haemolytic disorders and glycolysis will first be discussed. Breakdown of glucose follows two pathways. Both of these have several names, but they will here be called the Embden-Meyerhof pathway and the pentose phosphate shunt (Fig. 1). Normally about 10% of the glucose is metabolized through the pentose phosphate shunt [63]. A derivative of vitamin B<sub>1</sub>, thiamine diphosphate, is involved in the catabolism along this shunt [94]. In some of the steps an oxidation or a

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Glucose

Embden-Meyerhof pathway

Fig. 1. Erythrocyte metabolism. TPN and DPNH are reduced and

reduced co-factors called TPN and DPNH. There are several abbreviations for the structure of a substrate and TPN and DPNH are factors where the Embden-Meyerhof pathway is summed there is glycolysis. Work on their normal enzymology and sphere of enter

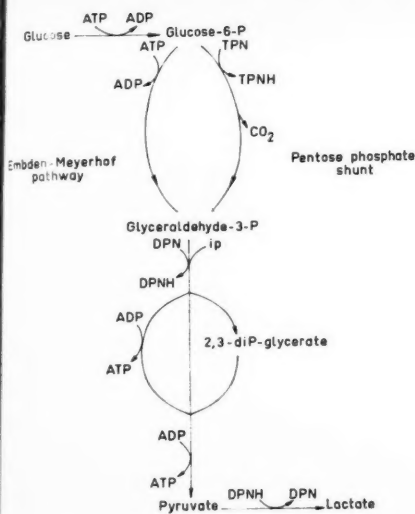


Fig. 1. A simplified scheme of glycolysis in the erythrocyte. Abbreviations: ATP = adenosine triphosphate; ADP = adenosine diphosphate; TPN and TPNH = triphosphopyridine nucleotide in oxidized and reduced form; DPN and DPNH = diphosphopyridine nucleotide in oxidized and reduced form; IP = inorganic phosphate; P = phosphate.

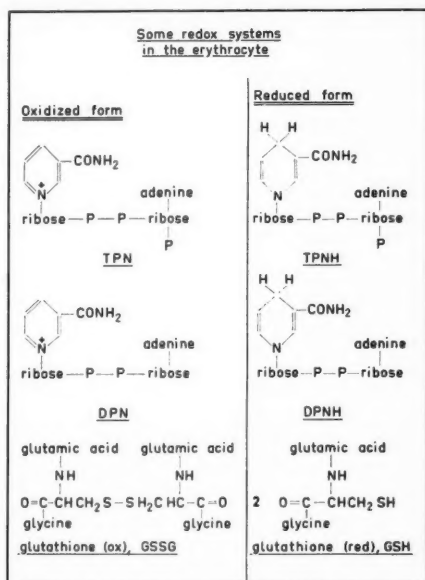


Fig. 2. Main structure of TPN, DPN, and glutathione in reduced and oxidized form.

reduction takes place and these steps are catalysed by enzymes to which co-factors, called pyridine nucleotides, are coupled. There are two kinds of pyridine nucleotides, abbreviated DPN and TPN. Their main structure is shown in Fig. 2. If they oxidize a substrate, they become reduced to DPNH and TPNH respectively. TPN is the co-factor in the pentose phosphate shunt, whereas DPN systems are only at work in the Embden-Meyerhof pathway. In this pathway the DPNH first formed is consumed in the final transfer to lactate, so there is no net synthesis in the completed glycolysis reaction.

Work done by Altman and Pranker and their associates [5, 6, 69] has shown an abnormality, probably a deficiency of a single enzyme in the glycolytic chain, in hereditary spherocytosis. Labelled inorganic phosphate enters the cells and is metabolized more slowly

there than in normal red cells. Addition of fluoride exaggerates the difference. This finding and certain other evidence would suggest that the deficient enzyme could be phosphofructokinase or enolase.

Some few investigations have also been made to clarify the defect in congenital non-spherocytic haemolytic anemias. Probably here too the explanation may be inborn errors of metabolism, but the sites of these errors are probably different from the site in hereditary spherocytosis.

In the red cells the TPN is mainly present in the reduced form, TPNH. This means that the content of TPN will normally determine the rate of degradation along the pentose phosphate shunt. The pyridine nucleotide systems are generally in equilibrium with other reduction-oxidation systems in the erythrocyte (Fig. 2), namely the glutathione system, which is present in high content, and

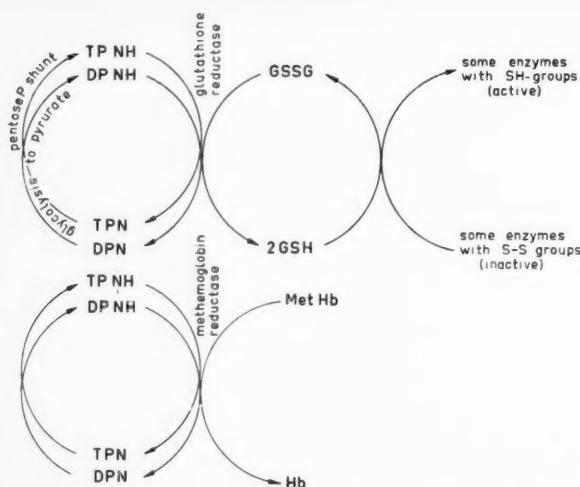


Fig. 3. Interaction of some oxidation-reduction systems in the erythrocyte.

the haemoglobin-methaemoglobin system. Some vital enzymes [71, 75] are active only in a reduced state, and it is important for them that the equilibrium of glutathione is shifted towards the reduced form (Fig. 3). If oxidized glutathione or methaemoglobin is present, it will oxidize TPNH to TPN and thus accelerate the reactions of the pentose phosphate shunt.

Investigations have shown that about 7% of an American Negro population have deficient activity of the first enzyme in the pentose phosphate shunt. The enzyme is called glucose-6-phosphate dehydrogenase, and its activity is reduced to about one-quarter to one-tenth of the normal value [e.g. 37]. Similar enzyme deficiency has also been found to a lesser extent in Caucasian populations. The subjects show no clinical signs except after taking certain drugs such as primaquine, sulphanilamide, or naphthalene, or after eating fava beans, when they exhibit a haemolytic disorder, often with methaemoglobinaemia and Heinz bodies [6]. These substances are thought to inhibit the enzyme glucose-6-phosphate dehydrogenase [23], thus blocking the pentose phosphate shunt and the TPNH production. The en-

zyme defect can be discovered either by direct enzyme determination or by estimation of the ability of the cells to keep glutathione in a reduced state after treatment with acetyl-phenyl-hydrazine, the so-called glutathione stability test. An Iranian family has also been described in which the members had no measurable amounts of glucose-6-phosphate dehydrogenase in their red blood corpuscles [57]. These patients suffered from spontaneous episodes of haemolytic activity and increased osmotic fragility.

Vitamin K catalyses the oxidation of TPNH to TPN, thus accelerating the pentose phosphate shunt. In this case the increased breakdown of glucose along the shunt is not accompanied by an increased net production of TPNH. The enzymes of the shunt are present in the red blood corpuscles in higher concentrations in the newborn infant than they are later. Both the increase in TPN and the elevated concentrations of the enzymes of the shunt contribute to increase the catabolism of glucose along the shunt. Especially in the newborn infant, with its physiological hypoglycaemia, this may lead to deficiency of glucose in some parts of the circulation. Both factors the



lack of glucose and the presence of vitamin K, thus tend to diminish the content of TPNH in the red cells, which may be fatal for the cells and cause haemolysis [97].

In paroxysmal nocturnal haemoglobinuria (PNH) the haemolytic disorder is probably not due to an error in glycolysis. The findings of a low content of the enzyme acetylcholinesterase [7] and a low content of the lipid lecithin [70] would suggest an error in lipid metabolism. The membrane of the erythrocyte is said to be a mosaic of lipids and proteins, and a fault in the membrane would therefore be the most likely explanation for this haemolytic disorder.

Few investigations have been made on the metabolism of the red cells coated with antibodies [1, 36, 86], and more work needs to be done before it is possible to interpret the results obtained.

It is likely in genetic disorders that some degree of the enzyme deficiency exists in all cells of the organism, but cells with specialized metabolism would probably suffer more from a metabolic block owing to the limited chances of bypassing the block or of producing essential metabolites via other routes. This is the case in erythrocytes, where ATP and reduced substances such as TPNH can only be formed by the breakdown of glucose to lactate or pyruvate. In the erythrocyte the ATP is essential for the transport of ions [24, 68] and thus for maintaining the right internal environment, and the TPNH is used for keeping some enzymes in their reduced, active state. This may explain why genetic disorders and other conditions affecting glycolysis produce the clinical sign of haemolysis.

#### Stig Sjölín:<sup>1</sup> Abnormal haemoglobins, pathogenesis, and diagnosis

In 1949 Pauling, Itano, Singer & Wells demonstrated the presence of an abnormal

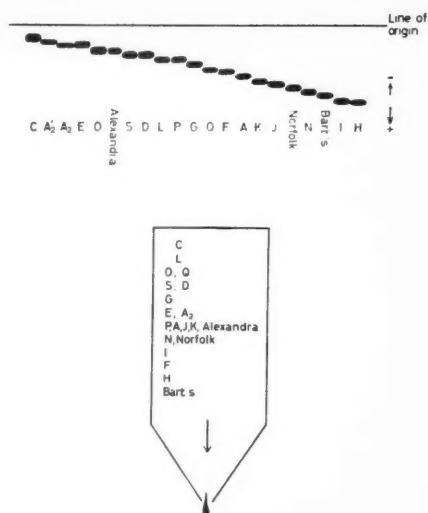


Fig. 4. Upper part: mobilities on paper electrophoresis at pH 8.6 of carboxyhaemoglobins. Lower part: sequence of separation of haemoglobins in an Amberlite IRC-50 ion-exchange column at pH 6.2 (the diagrams are taken from DACIE, *The Haemolytic Anaemias*, Part 1, pp. 292, 293, 1960).

haemoglobin in sickle-cell anaemia [65]. This haemoglobin was called Hb-S. In 1950 Hb-C was described, in 1951 Hb-D, and in 1954 Hb-E. Between 1954 and 1958 nine new haemoglobins were found, and lettered from H to Q. Since then at least seven probably new haemoglobins have been reported.

A child who has received the normal Hb-A from one of his parents and an abnormal haemoglobin from the other represents the heterozygous state. This condition is referred to as a trait. As a rule the heterozygous state is clinically harmless.

When both parents transmit the same abnormal haemoglobin to the child, the condition represents the homozygous state. The term "disease" is used to describe a condition produced by one or two haemoglobin variants in the absence of Hb-A. Several of these haemoglobin diseases are characterized by

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clinical and haematological signs, sometimes severe. In some of them increased red-cell destruction is a typical finding. In Hb-S disease, or sickle-cell anaemia, the life-span of the red cells is considerably diminished. The combinations of Hb-S with thalassaemia, Hb-C, or Hb-D lead to clinically significant excess haemolysis, although this is less severe than in Hb-S disease. In Hb-C disease the life-span of the red cells is moderately reduced. In Hb-D and Hb-E disease and in Hb-E/thalassaemia the red-cell survival is only slightly shortened.

The most important diagnostic methods used to detect the haemoglobinopathies are paper, starch block, or agar gel electrophoresis, chromatographic separation, resistance to alkali, and solubility measurements. The relative mobilities of the different haemoglobins at pH 8.6 on paper electrophoresis and on resin-column chromatography are seen in Fig. 4. Most, but not all abnormal haemoglobins can be separated by electrophoresis. In several cases, however, other methods are needed. Thus Hb-H and Hb-I, which show the same relative mobility on paper electrophoresis, can be differentiated by chromatography. Hb-S and Hb-D on the other hand show the same relative mobilities on paper electrophoresis and on chromatography, and can be differentiated only by their different solubilities. Reduced Hb-S has a very low solubility, while Hb-D is readily soluble.

As early as in 1950 Pauling and his collaborators were able to show that the difference between Hb-A and Hb-S lay in the globin part of the molecule [66]. During recent years Ingram has been able to explain the nature of the differences between some haemoglobins [46]. The only difference found between Hb-A and Hb-S was that one amino acid out of 300 forming the two polypeptide chains of a half molecule was substituted. One of the normal glutamic acid residues was replaced by valine. In Hb-C the same glutamic acid was also replaced, but this time by the amino acid lysine. And similar abnormalities have been demonstrated in some other haemoglobins. It is interesting

to note that such very minute molecular changes can result in marked physico-chemical differences and sometimes in severe disease.

It seems now also clear that these chemical alterations in the haemoglobin molecule are caused by mutation of one of two haemoglobin genes. The chemical nature of each of these genes is thought to determine the amino acid sequence of the corresponding polypeptide chain. It is now a generally accepted view that the anaemia of the haemoglobinopathies can arise in one of two ways. In Hb-S disease the basic cause seems to be an increased rate of haemolysis linked directly to the presence of sickled cells in the peripheral blood, and thus to the molecular abnormality of the haemoglobin. In the other haemoglobinopathies, which are associated with a diminished life-span of the red cells, there is no evidence of a direct connexion between the molecular abnormality and the diminished red-cell survival. Undoubtedly, however, an intracorporeal defect is to blame. In several haemoglobinopathies a slight retardation in the maximum rate of haemoglobin synthesis contributes to the anaemia.

Finally, I would like to stress that we may expect to find abnormal haemoglobins in patients from all over the world, not only among Negroes and in the Far East. Thus in Norfolk three members of a "pure" English family were found to be heterozygous carriers of a new haemoglobin not yet completely characterized [3]. At our department of paediatrics we have diagnosed Hb-H disease in two brothers [41]. And Wallenius & Höglund have since found haemoglobin H in an adult from Uppsala, who is not related to the two boys [90]. In Umeå Bergström & Jacobsson [8] have described several cases of an inherited non-haemolytic anaemia associated with an abnormal haemoglobin, as yet not identified. From Gothenburg Hansen and collaborators [40] have reported a family with hereditary cyanosis probably caused by the presence of an abnormal haemoglobin very similar to Hb-M of the Boston type.

### Lars Garby:<sup>1</sup> Determination of red-cell destruction

Previous speakers have discussed several different processes and disease states in the human organism that may prove deleterious to the red cells. These cells may suffer in that their ability to carry oxygen and carbon dioxide becomes impaired, but above all they may suffer in such a way that they die and disappear from the circulation before their normal life-span is ended.

No matter what the mechanism of the premature destruction of red cells may be, their mean life-span will be shortened. The effect of this upon the number of circulating cells will depend on how the organism can adapt itself to the new situation. The relation between the characteristics of practical interest is

$$D = \frac{N}{\bar{T}}$$

where  $D$  is the rate of red-cell destruction,  $N$  is the number of circulating red cells, and  $\bar{T}$  is their mean life-span. If the mean cell life-span is shortened, the number of circulating red cells would diminish unless the bone marrow could increase its production of red cells. In cases of chronic haemolytic anaemia the production has been shown to be increased by a factor of 6-7, and in anaemias of shorter duration by a factor of 2-3 [16, 31]. When the maximum production capacity of the bone marrow has been reached, the degree of anaemia is a direct measure of the severity of the haemolytic process.

In what follows, I shall try to comment shortly on some methods in use for assessing the degree of red-cell destruction.

First, a few words about the measurement of the behaviour of labelled red cells. There are two different types of labelling of the patients' own cells. In the first procedure, red cells are labelled during their formation, that is, a uniform age group of cells is labelled for instance with the help of radioactive iron. An example of the use of this meth-

od is illustrated by a case of chronic refractory anaemia published by Garby, Sjölin & Vahlquist [30]. This patient had a chronic refractory hypochromic anaemia with a normal chromium-survival curve, indicating that the great majority of the circulating red cells had a normal life-span. Since there was an anaemia with about 7 g% of peripheral haemoglobin, the rate of production of normal cells was reduced to about half its normal value. However, the facts that the bone marrow was hyperplastic and the rate of disappearance of the plasma iron was greatly increased indicated that the production of haemoglobin was normal or raised. The incorporation of iron into circulating red cells was slow, and reached a plateau at a value far below the normal range. These facts are probably best interpreted as reflecting the production of cells with very short survival, probably due to some intrinsic defect.

The second, more commonly used procedure of tracing the fate of labelled red cells involves labelling of peripheral red cells, that is, the circulating cell population is uniformly labelled with respect to the age of the cells. The mean life-span is obtained by drawing the tangent to the disappearance curve at zero time [59]. This tangent cuts the time axis at the mean cell life-span. In practice, red cells are labelled with radioactive chromium or with di-iso-propyl-fluoro-phosphonate (DFP) which is labelled in the phosphorus atom. In the case of the chromium method, there is a complication in that the chromium disappears from the circulation not only because of red-cell death but also due to elution of the chromium from the red cells. The chromium disappearance graph in a normal individual is therefore curved and not a straight line. As a measure of the chromium disappearance, that is, of the degree of haemolysis, the time taken to reach half the initial value is usually given. In normal individuals, this time is between 22 and 32 days [29], corresponding to a red-cell life-span of between 80 and 190 days. This means that the red-cell destruction must be about doubled if it is to be detected by the chromium method. An observation period of

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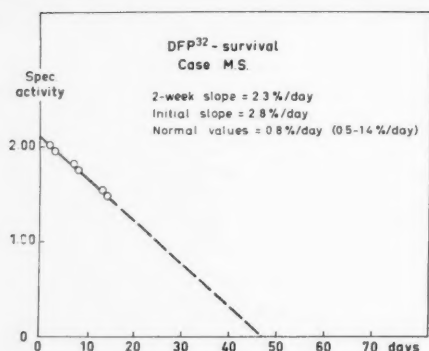


Fig. 5. Red-cell survival measured with  $\text{DFP}^{32}$  in a case of pancytopenia. Mean cell life span about 47 days.

about 4 weeks is necessary in order to achieve this precision. Di-iso-propyl-fluorophosphonate (DFP) is an organic phosphorus compound that binds irreversibly to certain proteins in the red cell [14]. It is not eluted *in vivo*, and gives straight lines in normal individuals with a red-cell life-span of 120 days. In our laboratory, this method has been used with the same precision as the chromium method with only about 2 weeks of observation. An example is shown in Fig. 5. This patient had had a pancytopenia for about 6 months, and the disappearance curve shows a relative rate of destruction of about 3 times the normal. The fact that there was anaemia with a haematocrit reading of about 20 implies that the absolute destruction rate was only about 1.5 times the normal, and that the capacity of the bone marrow to produce red cells was diminished.

It is a well established fact that the haem part of the haemoglobin molecule is not reutilized for new synthesis of haemoglobin. It is excreted in the form of urobilin and urobilinogen, and perhaps also in the form of dipyrroles [32, 33, 79]. It might therefore be expected that the excretion rate of such products could be used as an index of red-cell destruction. However, determinations of the urobilinogen excretion in normal subjects generally give values that are lower than the expected ones, and there are several dif-

ferent sources of error complicating the interpretation. The excretion should probably be related to the total haemoglobin mass. Values well above the upper limit of, say, 35 mg per 100 g of circulating haemoglobin can be taken as good evidence of increased red-cell destruction.

Another catabolic product of haem has received attention during recent years. It appears that normal man excretes small amounts of carbon monoxide through the lungs [83], and that this compound is formed when one of the methene bridges of haem is broken. If the excretion rate of carbon monoxide is increased, the alveolar carbon-monoxide concentration ought to rise. In fact, a rather good correlation has been demonstrated between the alveolar carbon-monoxide concentration and the degree of haemolysis as measured by the chromium method [26]. If this measurement can be put on a safe physiological basis, it will have definite advantages over methods that involve labelled cells, since it can be repeated, and only requires an observation time of a couple of hours.

It has been shown that under normal conditions only a very small proportion of red cells die intravascularly. In some haemolytic disorders there are reasons for believing that the number of red cells which die intravascularly is much larger. When free haemoglobin enters the circulation, it will form a complex with normally occurring plasma proteins called haptoglobins. The haemoglobin-haptoglobin complex is rapidly cleared from the plasma, presumably by way of the reticuloendothelial system in the liver, spleen, and bone marrow. The haptoglobin concentration in the plasma is lower the greater the rate of intravascular destruction. When the apparent half life is less than 17 days, using the  $\text{Cr}^{51}$  method, the haptoglobin concentration is virtually nil [64]. The large variation in haptoglobin levels between normal individuals, that is between some 30 and 180 mg %, limits the usefulness of the method.

An indication of the degree of haemolysis can also be obtained from measurements of

the red-cell production if the individual is in a steady state, that is, if the haematocrit readings are reasonably constant. The number of circulating reticulocytes has also been used for this purpose. Provided that the life-span of the circulating reticulocyte is constant, the number of circulating reticulocytes should be a good measure of the rate of red-cell production. Little is known about the life-span of the circulating reticulocyte under pathological conditions. Furthermore, the error of reticulocyte counts is usually rather large, so that in practice reticulocyte counts of at least 3-4 times the normal mean value must be required to establish with certainty that the rate of production is increased. Repeated counts by technicians not knowing the previous values should help the situation considerably. Reticulocyte counts should preferably be given in absolute numbers and not relative to the number of circulating red cells, since relative numbers may be misleading in cases of anaemia.

#### *Andreas Killander:*<sup>1</sup> Therapy

The therapeutic approach to the haemolytic anaemias is mostly empirical, and is only to a small extent based on results obtained with the specialized and refined diagnostic methods that are available. Regardless of the aetiology of the haemolytic disorder, one or more of the three main methods of treatment has to be employed. These methods comprise blood transfusions, cortisone or ACTH treatment, and splenectomy.

Blood transfusions may be life-saving in patients with rapid blood destruction and severe anaemia. Sometimes the presence of autoantibodies makes the matching of blood difficult. The patient's blood should therefore be genotyped before the first transfusion, and the specificity of his antibodies determined if possible. Except for emergencies, blood transfusions should be restricted to patients for whom no other treatment is possible. The aim should be not to achieve a

haemoglobin level as close as possible to normal, but to keep the patient at the lowest haemoglobin concentration that he can tolerate without suffering. This level varies with different patients and different ages. A haemoglobin level of about 7% seems to be sufficient in most children. Vigorous transfusion therapy depresses the activity of the bone marrow, and increases the risk of subsequent transfusion reactions due to isoimmunization, and in the long run also of haemosiderosis and haemochromatosis. For the same reason iron therapy should be refrained from unless there is evidence of iron deficiency. This is very rarely the case in haemolytic anaemia.

In newborn infants with congenital haemolytic anaemia the accumulation of bilirubin sometimes becomes so excessive as to require exchange transfusion. The indication for this procedure depends much more on the degree of hyperbilirubinaemia than on the haemoglobin level.

The beneficial effect of cortisone and its derivatives and of ACTH in a number of blood diseases is now well recognized, but the mode of action of these hormones has not yet been clarified. It is beyond the scope of this paper to try to evaluate the evidence for and against the different explanations which have been put forward. Let it suffice to say that there is some evidence that cortisone may suppress the production of antibodies. No effect has been demonstrated on the circulating antibodies. The initial doses should be high, and equivalent to 300-400 mg of cortisone in the adult. This corresponds to 60-80 mg of prednisone, which is at present the most widely used preparation. Treatment should be continued until the rate of haemolysis decreases, and then gradually reduced to the smallest amount necessary to keep the illness under control. If there is no effect within two weeks, it is improbable that the drug will work later on. It is uncertain whether ACTH may be helpful when cortisone fails.

The spleen is generally considered to be important in two ways. It is one of the sources of antibodies, and it removes damaged,

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spherocytic, and sensitized red cells from the circulation. The latter function is by far the most important in haemolytic anaemia. Following splenectomy in patients with autoimmune haemolytic anaemia little or no changes in the serological findings take place as a rule. In almost all patients splenectomy results in some lowering of the rate of haemolysis. If, however, the rate of haemolysis before the operation greatly exceeds the patient's powers of compensation, a small improvement in the red-cell life-span following splenectomy may be of little clinical benefit. On the other hand, if equilibrium between destruction and formation can be achieved or nearly achieved, removal of the spleen may lead to significant improvement. It is difficult or impossible to predict the result of splenectomy in such cases. Surface counting of radioactive chromium over the spleen and liver may provide a useful guide [20]. High radioactivity over the spleen compared with the liver indicates preferential destruction of the red cells in the spleen. This finding is usually associated with a favourable result of a subsequent splenectomy. A low relative splenic radioactivity has been found in many patients who subsequently failed to respond to splenectomy. However, there is not as yet enough information available to warrant the conclusion that all patients with a low relative accumulation of radioactivity in the spleen fail to respond to splenectomy.

In haemolytic anaemia secondary to other disease, splenectomy may be of value in selected cases. The risk of post-operative complications in this group is high, and the effect is often of doubtful value.

This leads us to consider other possible contraindications to splenectomy. It is known that agenesis of the spleen may be associated with increased susceptibility to infection [47]. In 1952 King & Schumacker [52] reported the occurrence of fatal infections in all of five infants who had undergone splenectomy at less than 6 months of age. Since then reports from other clinics have lent support to their observation [34, 83]. Contradictory results, however, have also

been reported [54, 58]. In a recent well-documented paper, Robinson & Sturgeon [72] report their experience of 110 splenectomized children followed up for a median period of 6 years. They divided their series into two groups. The first comprised 63 patients considered not to be predisposed to infections. One infant, 4 months old at the time of splenectomy, developed meningitis from which he recovered. The second group included patients with a variety of underlying disorders which were thought to predispose to infection. In this group of 47 patients, 12 developed severe infections after operation. From these results, and from findings recently reported by other workers, it is evident that splenectomy carries a very low risk of post-operative infection when carried out over the age of 6 months for the generally accepted indications of traumatic rupture of the spleen, hereditary spherocytosis, and idiopathic thrombocytopenic purpura. Since the possibility cannot be entirely excluded that splenectomy in early life may carry a small hazard, it seems reasonable to postpone the operation until at least beyond the limit of 6 or possibly 12 months.

In 1955 Dameshek published the observation of exacerbation of lupus erythematosus following splenectomy for idiopathic thrombocytopenic purpura and auto-immune haemolytic anaemia [21]. This report evoked a most controversial discussion, which is still going on. It appears that most workers in this field do not share Dameshek's view in this respect. And in fact it has been reported that haemolytic anaemia in overt lupus erythematosus has responded favourably to splenectomy [74].

Looking at the impressive list of different haemolytic disorders, it is obvious that no effective therapy is available for most of them. I should like therefore to restrict my comment to a few of these states. The haemolytic anaemia associated with virus infections, for example, primary atypical pneumonia and mononucleosis, may be severe, but it is always a self-limited disease. Transfusions may be used to tide the patient over



the dangerous period. Prednisone has been used with good results, and without any apparent aggravation of the virus infection.

The most rewarding results of splenectomy are obtained in hereditary spherocytosis. The results are so consistently favourable that a therapeutic failure indicates either that the initial diagnosis was wrong or that accessory spleens are present. It is difficult to make a specific recommendation concerning the best time to perform splenectomy. Jaundice, the presence of gallstones, and anaemia requiring regular transfusions may be considered absolute indications at any age. Unless the patient's illness is completely compensated and the patient quite symptom-free, it would seem wise to remove the spleen before the age of 7-9 years, after which the incidence of gallstones has been reported to increase rapidly. In congenital non-spherocytic haemolytic anaemia corticosteroids are without effect. Some cases will derive benefit from splenectomy.

In thalassaemia major splenectomy has been reported to lessen the transfusion requirement in selected cases. Patients with sickle-cell anaemia as a rule seem to derive little benefit from splenectomy.

Auto-immune haemolytic anaemia poses the most difficult problems with regard to

therapy. The aim of treatment in this state is to keep the patient alive until a temporary or lasting remission occurs. It has been shown in large series collected by, for example, Dacie and associates in London [11], and Dausset's group in Paris [22], that the cure rate is about 50%.

Unfortunately, it is impossible to predict which patients are going to recover. The therapeutic approach consists of initial treatment with high doses of prednisone or ACTH. If the patient fails to respond, or if there are complications or contraindications to this therapy, splenectomy should be considered. As mentioned before, investigation of the accumulation of radioactive chromium in the spleen and liver is desirable but is not of crucial importance for determining whether splenectomy should be performed. If splenectomy is unsuccessful, prednisone or ACTH may be tried again. If they fail once more, which is probable, the patient is in a sad situation. There remain only blood transfusions, which at this stage are frequently complicated by reactions. These may be diminished by using washed red-cell concentrate instead of whole blood. Although the prognosis for these patients is poor, it is not without hope. There is always the chance that the disease will die before the patient.

### References

1. ABRAHAMOV, A. and DIAMOND, L. K.: Erythrocyte glycolysis in erythroblastotic newborns. *Amer J Dis Child*, 99: 202, 1960.
2. ADNER, P. L. and SJÖLIN, S.: Unexpected blood group incompatibility, revealed by <sup>51</sup>Cr-labelled red cells. *Scand J Clin Lab Invest*, 9: 265, 1957.
3. AGER, J. A. M., LEHMANN, H. and VELLA, F.: Haemoglobin 'Norfolk': a new haemoglobin found in an English family. *Brit Med J*, ii: 539, 1958.
4. ALLISON, A. C.: Acute haemolytic anaemia with distortion and fragmentation of erythrocytes in children. *Brit J Haemat*, 3: 1, 1957.
5. AITMAN, K. J., TABECHIAN, H. and YOUNG, L. E.: Some aspects of the metabolism of red blood cells from patients with hemolytic anemias. *Ann NY Acad Sci*, 75: 142, 1958.
6. AITMAN, K. J.: Some enzymatologic aspects of the human erythrocyte. *Amer J Med*, 27: 936, 1959.
7. AUDITORE, J. V., HARTMANN, R. C. and COLE, E. F.: Potassium transport in the acetylcholinesterase-deficient erythrocytes of paroxysmal nocturnal hemoglobinuria (PNH). *J Clin Invest*, 38: 702, 1959.
8. BERGSTRÖM, I. and JACOBSSON, L.: Hereditär benign erythroretikulos. *Svensk Läkartidn*, 56: 1195 1959.
9. BEUTLER, E.: The hemolytic effect of primaquine and related compounds: a review. *Blood*, 14: 103, 1959.
10. BRAFIELD, A. J. and REEF, I.: Atypical congenital hemolytic anemia. *Arch Dis Child*, 32: 93, 1957.
11. CHERTKOW, G. and DACIE, J. V.: Results of splenectomy in auto-immune haemolytic anaemia. *Brit J Haemat*, 2: 237, 1956.
12. CLÉMENT, R., BERNARD, J., PAPAIOANNOU,

- A., MATHÉ, G., HABIB, R. and DESIGNOLLE, M.: Purpura thrombotique thrombocytopénique (maladie de Moschcovitz) chez une enfant de dix mois. *Presse Méd.*, 65: 2144, 1957.
13. COCK, TH. C.: Acute hemolytic anemia in neonatal period. *Amer J Dis Child*, 94: 77, 1957.
  14. COHEN, J. A. and WARRINGA, M. G. P. J.: Fate of  $P^{32}$  labelled diisopropylfluorophosphate in human body and its use as a labelling agent in study of turnover of blood plasma and red cells. *J Clin Invest*, 33: 459, 1954.
  15. COOMBS, R. R. A. and ROBERTS, G. FULTON: Antiglobulin reaction. *Brit Med Bull*, 15: 113, 1959.
  16. CROSBY, W. H. and AKEROYD, J. H.: The limit of hemoglobin synthesis in hereditary hemolytic anemia. *Amer J Med*, 13: 273, 1952.
  17. DACIE, J. C.: Differences in the behaviour of sensitized red cells to agglutination by antiglobulin sera. *Lancet*, ii: 954, 1951.
  18. DACIE, J. V.: *The Haemolytic Anaemias: Congenital and Acquired*. Churchill Ltd., London, 1954.
  19. — *The Haemolytic Anaemias. Part I. The Congenital Anaemias*. 2nd edition, Churchill Ltd., London, 1960.
  20. — *Acquired haemolytic anaemias*. *Brit Med Bull*, 15: 67, 1959.
  21. DAMESHEK, W.: Hypersplenism. *Bull NY Acad Med*, 31: 133, 1955.
  22. DAUSSET, J. and COLOMBANI, J.: The serology and the prognosis of 128 cases of autoimmune hemolytic anemia. *Blood*, 14: 1280, 1959.
  23. DESFORGES, J. F., KALAW, E. and GILCHRIST, P.: Inhibition of glucose-6-phosphate dehydrogenase by hemolysis-inducing drugs. *J Lab Clin Med*, 55: 757, 1960.
  24. DUNHAM, E. T. and GLYNN, I. M.: Adenosinetriphosphatase activity and ion movements. *J Physiol*, 152: 61P, 1960.
  25. DUNSFORD, I. and GRANT, J.: *The Antiglobulin (Coombs) Test in Laboratory Practice*. Oliver and Boyd, Edinburgh-London, 1959.
  26. ENGSTEDT, L.: Endogenous formation of carbon monoxide in hemolytic disease. *Acta Med Scand*, 159: Suppl. 332, 1957.
  27. ERLANDSON, M. E. and HILGARTNER, M.: Hemolytic disease in the neonatal period and early infancy. *J Pediat*, 54: 556, 1959.
  28. EVANS, R. S. and WEISER, R. S.: The serology of autoimmune hemolytic disease: observations on forty-one patients. *Arch Int Med*, 100: 371, 1957.
  29. FOCONI, S. and SJÖLIN, S.: Survival of  $Cr^{51}$ -labelled red cells from new-born infants. *Acta Paediat*, 48: Suppl. 117: 18, 1959.
  30. GABBY, L., SJÖLIN, S. and VAHLQUIST, B.: Chronic refractory hypochromic anemia with disturbed haem-metabolism. *Brit J Haemat*, 3: 55, 1957.
  31. GIBLETT, E. R., COLEMAN, D. H., PARZIOBIROLI, G., DONOHUE, D. M., MOTULSKY, A. G. and FINCH, C. A.: Erythrokinetics: Quantitative measurements of red cell production and destruction in normal subjects and patients with anemia. *Blood*, 11: 291, 1956.
  32. GILBERTSEN, S., HAWKINSON, V. and WATSON, C. J.: Studies of the dipyrromethene ("Fuscin") pigments. II. The contrasting ratios and significance of the fecal urobilinogen and mesobilifuscin in certain anemias. *J Clin Invest*, 38: 1175, 1959.
  33. GILBERTSEN, S., LOWRY, P. T., HAWKINSON, V. and WATSON, C. J.: Studies of the dipyrromethene ("Fuscin") pigments. I. The anabolic significance of the fecal mesobilifuscin. *J Clin Invest*, 38: 1166, 1959.
  34. GOFSTEIN, R. and GELLIS, S. S.: Splenectomy in infancy and childhood. *Amer J Dis Child*, 91: 566, 1956.
  35. GREEN, N. and GOLDENBURG, H.: Acute hemolytic anemia and hemoglobinemia complicating infectious mononucleosis. *Arch Int Med*, 105: 108, 1960.
  36. GREENWALT, T. J. and AYERS, V. E.: Phosphate partition in the erythrocytes of normal newborn infants and infants with erythroblastosis fetalis. II. Quantitative paper chromatography. *Blood*, 15: 698, 1960.
  37. GROSS, R. T., HURWITZ, R. E. and MARKS, P. A.: An hereditary enzymatic defect in erythrocyte metabolism: glucose-6-phosphate dehydrogenase deficiency. *J Clin Invest*, 37: 1176, 1958.
  38. GRUBB, R.: Agglutination of erythrocytes coated with "incomplete" anti-Rh by certain rheumatoid arthritic sera and some other sera. The existence of human serum groups. *Acta Path Microbiol Scand*, 39: 195, 1956.
  39. GRUCHY DE, G. C.: *Clinical Haematology in Medical Practice*. Blackwell, Oxford, 1958.
  40. HANSEN, H. A., JAGENBURG, O. R. and JOHANSSON, B. G.: Studies on an abnormal hemoglobin causing hereditary congenital cyanosis. *Acta Paediat*, 49: 503, 1960.
  41. HEDENBERG, F., MÜLLER-EBERHARD, U., SJÖLIN, S. and WRANNE, L.: Haemoglobin H and inclusion-body anaemia in a Swedish family. *Acta Paediat*, 47: 652, 1958.
  42. HOLLÄNDER, L. and BATSCHELET, E.: Beitrag zur Spezifität antierythrocytärer Autoantikörper. Proc 6th Congress Europ Soc Haemat, p. 680. Copenhagen 1957.
  43. HOWELL, O. S., MALCOLM, J. M. and PIKE, R.: The FII-agglutinating factors in serum of patients with non-rheumatic diseases. *Amer J Med*, 29: 662, 1960.



44. HUGHES-JONES, N. C., MOLLISON, P. L. and VEALL, N.: Removal of incompatible red cells by the spleen. *Brit J Haemat*, 3: 125, 1957.
45. HÖGMAN, C., KILLANDER, J. and SJÖLIN, S.: A case of idiopathic autoimmune haemolytic anaemia due to anti-e. *Acta Paediat*, 49: 270, 1960.
46. INGRAM, V. M.: Chemistry of the abnormal human haemoglobins. *Brit Med Bull*, 15: 27, 1959.
47. IVERMARK, B. I.: Implications of agenesis of the spleen on the pathogenesis of conotruncus anomalies in childhood. *Acta Paediat*, 44: Suppl. 104, 1955.
48. JANDL, J. H., JONES, R. and CASTLE, W. B.: The destruction of red cells by antibodies in man. I. Observations on the sequestration and lysis of red cells altered by immune mechanisms. *J Clin Invest*, 36: 1428, 1957.
49. JANDL, J. H. and TOMLINSON, A. S.: The destruction of red cells by antibodies in man. II. Pyrogenic, leucocytic and dermal responses to immune hemolysis. *J Clin Invest*, 37: 1202, 1958.
50. JANDL, J. H. and KAPLAN, M. E.: The destruction of red cells by antibodies in man. III. Quantitative factors influencing the patterns of hemolysis *in vivo*. *J Clin Invest*, 39: 1145, 1960.
51. JENSEN, K. G.: Elution of incomplete antibodies from red cells. A comparison of different methods. *Vox Sang*, 4: 230, 1959.
52. KING, H. and SCHUMACKER, H. B., JR.: Splenic studies. I. Susceptibility to infection after splenectomy performed in infancy. *Ann Surg*, 136: 239, 1952.
53. KRIVIT, W., SMITH, R. T., MARVIN, J. F., READ, R. and GOOD, R. A.: Congenital nonspherocytic hemolytic anemia: Two non-familial cases with red cell survival studies. *J Pediat*, 49: 245, 1956.
54. LASKI, B. and MACMILLAN, A.: Incidence of infection in children after splenectomy. *Pediatrics*, 24: 523, 1959.
55. LASKI, B., WAKE, E., GUNSON, H. and BAIN, H. W.: Idiopathic acquired hemolytic anaemia in two young infants. *Amer J Dis Child*, 100: 524, 1960.
56. LELONG, M., ALAGILLE, D. and DORMONT, J.: La forme icterique néonatale de la maladie de Minkowski-Chauffard. *Arch Franc Pédiat*, 16: 577, 1959.
57. LÖHR, G. W. and WALLER, H. D.: Hämo-lytische Erythrocytopathie durch Fehlen von Glukose-6-Phosphatdehydrogenase in roten Blutzellen als dominant vererbliche Krankheit. *Klin Wschr*, 36: 865, 1958.
58. McKINNON, W. M. P., BOLEY, S. J. and MANPUL, J.: Infection in children following splenectomy for traumatic rupture. *Amer J Dis Child*, 98: 710, 1959.
59. MILLS, J. N.: The life span of the erythrocyte. *J Physiol*, 105: 16 (P), 1946.
60. MOLLISON, P. L.: *Blood Transfusion in Clinical Medicine*. 2nd edition, Blackwell Scientific Publications, Oxford, 1956.
61. — Red cell survival in haemolytic syndromes. *Brit Med Bull*, 15: 59, 1959.
62. — Blood-group antibodies and red-cell destruction. *Brit Med J*, ii: 1036, 1123, 1959.
63. MURPHY, J. R.: Erythrocyte metabolism. II. Glucose metabolism and pathways. *J Lab Clin Med*, 55: 286, 1960.
64. NOSSLIN, B. F. and NYMAN, M.: Haptoglobin determination in diagnosis of haemolytic diseases. *Lancet*, i, 1000, 1958.
65. PAULING, L., ITANO, H. A., SINGER, S. J. and WELLS, I. C.: Sickle cell anemia, a molecular disease. *Science*, 110: 543, 1949.
66. PAULING, L., ITANO, H. A., WELLS, I. C., SCHROEDER, W. A., KAY, L. M., SINGER, S. J. and COREY, R. B.: Sickle cell anemia hemoglobin. *Science*, 111: 459, 1950.
67. PONT, M. E., JONES, J. A. and BULEN, J. A.: Exchange transfusion in a neonatal crisis of congenital hemolytic anemia. *Amer J Dis Child*, 99: 231, 1960.
68. POST, R. L., MERRITT, C. R., KINSOLVING, C. R. and ALBRIGHT, C. D.: Membrane adenosine triphosphatase as a participant in the active transport of sodium and potassium in the human erythrocyte. *J Biol Chem*, 235: 1796, 1960.
69. PRANKERD, T. A. J.: Inborn errors of metabolism in red cells of congenital hemolytic anemias. *Amer J Med*, 22: 724, 1957.
70. PRANKERD, T. A.: Red-cell structure and metabolism in haemolytic anaemia. *Brit Med Bull*, 15: 54, 1959.
71. RAPOPORT, S. M.: Glutathione stability and pyrophosphatase activity in reticulocytes: Direct evidence for the importance of glutathione for the enzyme status in intact cells. *Nature*, 186: 967, 1960.
72. ROBINSON, T. W. and STURGEN, P.: Post-splenectomy infection in infants and children. *Pediatrics*, 25: 941, 1960.
73. ROSENFELD, R. E., HABER, G. V. and GILBERT, H. S.: Complement and the antiglobulin test. *Vox Sang*, 5: 182, 1960.
74. SARLES, H. E. and LEVIN, W. C.: The role of splenectomy in the management of acquired autoimmune hemolytic anemia complicating systemic lupus erythematosus. *Amer J Med*, 26: 547, 1959.
75. SCHEUCH, D. and RAPOPORT, S. M.: Glutathione and Triosephosphatdehydrogenase-Aktivität in roten Blutzellen von Kaninchen. *Klin Wschr*, 38: 757, 1960.
76. SELWYN, J. G. and DACIE, J. V.: Autohemolysis and other changes resulting from the incubation in vitro of red cells from patients with congenital hemolytic anemia. *Blood*, 9: 414, 1954.

77. SHAHIDI, N. T. and DIAMOND, L. K.: Enzyme activity in erythrocytes in congenital nonspherocytic hemolytic anemia. *Pediatrics*, 24: 245, 1959.
78. SHAPIRO, CH. M., JOSEPHSON, A. M., ROZENGVAIG, S. and KAUFFMAN, A.: Hereditary spherocytosis in neonatal period: Diagnosis, incidence and treatment. *J Pediat*, 50: 308, 1957.
79. SIEDEL, W., STICH, W. and EISENREICH, F.: Promesobilifuscin (Mesobilileukan), ein neues physiologisches Abbauprodukt des Blutfarbstoffes. *Naturwissenschaften*, 35: 316, 1948.
80. SJÖLIN, S.: The resistance of red cells in vitro. A study of the osmotic properties, the mechanical resistance and the storage behaviour of red cells of fetuses, children and adults. *Acta Paediat*, 43: Suppl. 98, 1954.
81. SJÖLIN, S., ADNER, P. L. and FOCONI, S.: <sup>51</sup>Cr-labelled red cells as a means of detecting small quantities of antibodies. Proc. 7th Congr. Europ. Soc. Haemat., London 1959, part II, 1129, 1960.
82. SJÖLIN, S.: Studies on osteopetrosis. II. Investigations concerning the nature of the anaemia. *Acta Paediat*, 48: 529, 1959.
83. SJÖSTRAND, T.: Endogenous formation of carbon monoxide in man. *Nature*, 164: 580, 1949.
84. SMITH, C. H., ERLANDSON, M., SCHULMAN, I. and STARN, G.: Hazard of severe infections in splenectomized infants and children. *Amer J Med*, 22: 390, 1957.
85. STEWART, J. W. and MOLLISON, P. L.: Rapid destruction of apparently compatible red cells. *Brit Med J*, i: 1274, 1959.
86. STORTI, E., VACCARI, F. and BALDINI, E.: Changes in red cells metabolism in presence of incomplete antibodies. *Experientia*, 12: 108, 1956.
87. TODD, R. McLAREN and O'DONOHUE, N. V.: Acute acquired hemolytic anemia associated with herpes simplex infection. *Arch Dis Child*, 33: 524, 1958.
88. TROUP, S. B., SWISHER, S. N. and YOUNG, L. E.: The anemia of leukemia. *Amer J Med*, 28: 751, 1960.
89. TUFFY, PH., BROWN, A. K. and ZUELZER, W. W.: Infantile pyknoctosis. *Amer J Dis Child*, 98: 227, 1959.
90. WALLENIUS, G. and HÖGLUND, N. J.: Personal communication.
91. WEINER, W., BATTEY, D. A., CLEGHORN, T. E., MARSON, F. G. W. and MEYNELL, M. J.: Serological findings in a case of haemolytic anaemia with some general observations on the pathogenesis of this syndrome. *Brit Med J*, 2: 125, 1953.
92. WILE, S. A. and STURGEON, PH.: Thrombotic thrombocytopenic purpura: Review of subject with report of three cases in children. *Pediatrics*, 17: 882, 1956.
93. WINTROBE, M. M.: *Clinical Hematology*. Fourth edition. Lea & Febiger, Philadelphia, 1956.
94. WOLFE, J. W., BRIN, M. and DAVIDSON, C. S.: The effect of thiamine deficiency on human erythrocyte metabolism. *J Clin Invest*, 37: 1476, 1958.
95. ZETTERSTRÖM, R. and STRINDBERG, B.: Sporadic congenital spherocytosis associated with congenital hypoplastic thrombocytopenia and malformations. *Acta Paediat*, 47: 14, 1958.
96. ZINKHAM, W. H. and CHILDS, B.: Defect of glutathione metabolism in erythrocytes from patients with naphthalene-induced hemolytic anemia. *Pediatrics*, 22: 461, 1958.
97. ZINKHAM, W. H.: An in-vitro abnormality of glutathione metabolism in erythrocytes from normal newborns: mechanism and clinical significance. *Pediatrics*, 23: 18, 1959.
98. ZINKHAM, W. H. and LENHARD, R. E., JR.: Metabolic abnormalities of erythrocytes from patients with congenital nonspherocytic hemolytic anemia. *J Pediat*, 55: 319, 1959.

Submitted April 8, 1961

Accepted April 11, 1961

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SUMMARY OF SUPPLEMENTS

## Pneumoencephalography in Atrophic Brain Lesions in Infancy and Childhood

by J. C. MELCHIOR

(Supplement 127)

The purpose of the present study is to examine the prognostic value of pneumoencephalography (P.E.G.) in a number of childhood disorders causing either real or apparent dilatation of the ventricular system. The value of certain measuring methods is examined and the reliability of one such method is ascertained.

A survey of the literature on P.E.G. is made, and this is divided into four parts. The first reviews its development with special reference to experimental examinations, results and complications in adults. In the second part reports concerning paediatric examinations are mentioned, and in the third the development of P.E.G. in Denmark is briefly reviewed. The picture revealed is that of a tendency for interest to fluctuate over the 40 years. At the beginning excessive enthusiasm was resisted strongly. In the next ten years there was little critical evaluation of the examination but this was followed by increasing interest in factors influencing complications. In the last ten years the use of air insufflation has increased, in other fields as well as in tumour diagnosis and there has been a more critical evaluation of the results obtained. In the fourth

section the different measurements which are used to evaluate the degree of dilatation of the ventricular system are discussed. These methods are divided into planimetric and linear measurements and are given as exact or relative values. The P.E.G. measurements in suitable cases can be compared with autopsy material. The most frequently used figures are Evans' ratio and Schiersmann's index and these are discussed in detail, and it is emphasised that the use of relative values are of particular importance in childhood where the exact figures vary very much.

The terminology and abbreviations used in the text are discussed. The present material is made up of 271 children (171 boys and 100 girls). To be included in the material the following criteria had to be fulfilled:

- (i) All patients were under fifteen years of age at the time of examination in the Neurosurgical Department.
- (ii) Tumours and acute infections of the C.N.S. were excluded.
- (iii) P.E.G. was performed either in the Neurosurgical Department or immediately before admission.

- (iv) At least one lateral ventricle on P.E.G. was greater than a quarter of the distance from the ventricular system's midline to the internal cranial wall (a ratio of 0.25 or more).
- (v) Admission was before July 1955 and therefore the shortest observation period was a year.

At the time of admission 74 children were less than a year old and 126 less than two years. The symptoms fell mainly into three groups: (a) Motor handicap, (b) Mental retardation and (c) Convulsions. In 158 patients the disease was recognised before the age of one year. As far as aetiological factors are concerned, familial predisposition accounts for 10 %, prenatal damage for 15 %, perinatal damage for 40 % and postnatal factors for approximately 40 %. About 40 % of the cases suggest multiple aetiology.

The evaluation of P.E.G. is only attempted when there is adequate filling of the ventricular system. The internal cranial width and the greatest width of the anterior horns are measured and from this Evans' ratio is calculated. The same measurement is performed on each side and a ratio obtained for the right and left side. At the same time information on possible displacement of the ventricular system's midline is obtained. The width of the third ventricle is measured and the amount of cortical air assessed but no exact measurement is made. Finally, findings of special significance are reported.

As a result of these measurements the material is divided into five major types of ventricular dilatation:

- (a) Bilateral dilatation with equal ratios without displacement.

- (b) Bilateral dilatation with equal ratios with at least 5 mm displacement
- (c) Bilateral dilatation with different ratios on each side but without displacement.
- (d) Bilateral dilatation with different ratios on each side with displacement. The difference in ratio has to be more than 0.05 before being considered significant.
- (e) This group is made up of few patients and consists of those with only one lateral ventricle ratio of 0.25 or more.

More than 50 % are in the first group where the dilatation is almost symmetrical.

The left lateral ventricle is more frequently dilated than the right. The third ventricle varies in size from 2-26 mm.

A febrile episode of a few days' duration is present in approximately 70 % of the children after insufflation. More serious complication such as latent or manifest shock, marked neck and back stiffness etc. are present in only a few. Two deaths occurred within twenty-four hours of insufflation. Both children had relatively large third ventricles but only moderate dilatation of the lateral ventricles. The mortality rate is low especially when one considers the number of children included in the material who are already very ill at the time of the examination (58 of the 271 patients had died at the time of follow-up).

Finally, mention is made of the skull X-rays and electroencephalography.

The follow-up examination includes all 271 patients, and most of them were personally examined by the author. The patient's condition is assessed from two standpoints: (1) Evaluation of the patient's condition at the time of follow-up.

i.e. whether he is healthy, has symptoms, or whether he is under institutional care or dead and (2) An evaluation based on the changes which have taken place in the patient's condition between the time of admission and the time of follow-up. In this latter category the findings are classified as improved, unchanged or worse.

Independent of the method of evaluation, it is shown that as far as the degree of dilatation of the ventricular system is concerned, the results as expressed in terms of ratio are the same. The smaller the ratio is, the better the prognosis. In the group with a ratio under 0.30 approximately 80 % have done well, and in the group with a ratio of 0.50 or more, more than 80 % are under care or have died.

It is shown that it makes little difference whether Evans' ratio or Schiersmann's index is used but the former method is superior in cases of displacement of the ventricular system or unequal ratio.

Unilateral dilatations have a better prognosis than other types.

If the ratios are unequal the prognosis is dependent on the ratio difference and the total degree of dilatation.

The presence of cortical air in great amounts has serious prognostic implications, but is better if the air is frontally placed compared to any other position. Bilateral changes have the worst prognosis, and this is also the case in children below one year of age, so that one cannot consider changes in this age group as merely accidental.

The width of the third ventricle is of definite prognostic value. Only a few patients with a third ventricle width of more than 8 mm have done well. With this measurement one uses exact figures and

these are related to age and cranial diameter. Almost all patients who have done well have third ventricles below 6 mm in diameter and cranial diameters greater than or corresponding to their age.

The period of observation has no bearing on the follow-up results.

The age at the time of examination shows a fairly uniform distribution of different types of P.E.G. The only specific finding on the older age groups is that there are not such severe degrees of dilatation. The age at the time of recognition of the disease seems to have a definite bearing on the prognosis. The later the disease is recognised, the better is the prognosis.

As might be expected, there is no definite relationship between symptoms and P.E.G. findings. In cases of mental retardation there is a correlation between the lower I.Q.'s and severe dilatation of the ventricular system.

The symptoms as well as their time of recognition are related to the follow-up results, and it is seen that the disorders which are recognised late, regardless of their character, have a better prognosis than those recognised earlier, with a definite dividing line at two years of age. This corresponds to the absence of high ratios in the older age groups.

The different aetiological factors are related to P.E.G. findings and to the follow-up examination. It is shown that patients with a familial predisposition have a somewhat worse prognosis than one would have expected from their ratios. The most severe changes and the poorest prognoses are linked with perinatal changes, whereas patients with postnatal damage frequently do well and often show asymmetrical and possibly unilateral dilatation.

Changes found on X-ray examination of the skull have no particular prognostic value. This is applicable to increased digital markings and deep posterior fossae. Changes in shape, however, often indicate a poor prognosis. This is true when the relationship between head circumference and prognosis is examined, but microcephaly is of much more serious prognostic value than macrocephaly.

E.E.G. shows the relationship between focal changes and non-symmetrical ventricular dilatation, but there is no particular relationship between cortical air findings and E.E.G. An abnormal E.E.G. under one year of age is a bad sign, whereas a normal E.E.G. in the same age group is not necessarily a good sign. Severely dilated third ventricles are most often associated with abnormal E.E.G.'s but not with a specific type.

Sixty-three patients have had more than one P.E.G. examination. This is considered an important method of evaluating the use of ratios. It is shown that there is a close correlation between the different P.E.G.'s. If the examinations are carried out within a short period of time there is almost no difference in the ratios obtained. If several years have elapsed the ratio is frequently the same even if there have been great changes in cranial diameter. In a number of cases the ratio has increased over the years, and this corresponds to a clinical deterioration. In no case is deterioration related to a diminishing dilatation. P.E.G.'s performed in other departments show the same close correlation and this

means that by using relative figures the ratio becomes independent of technical factors, e.g. distance from the X-ray apparatus etc., whereas if exact figures are quoted, it is essential to have detailed information concerning such factors.

Operations performed and the results thereof are mentioned briefly. Good results are obtained in cases of removal of meningo-cerebral scars. Congenital malformations of various kinds and more severe perinatal damage do not show the same good results. Nevertheless, some patients have had cysts removed and have shown improvement.

The pathological findings are discussed. The fact that cortical biopsies are often too small for detailed histological examination has in no case led one to suspect a more serious disorder which is not confirmed later. The material obtained at operation confirms the cortical and sub-cortical findings but reveals little about the ventricular system.

The patients who have died are reported and it is shown that the direct causes of death are frequently febrile disorders, pneumonia or bronchitis or, in a great many cases, unknown but possibly cerebral hyperpyrexia. In this connection attention is drawn to the possibility of anxiety reactions precipitating "Voodoo deaths."

Autopsies have been carried out on 22 of the 58 patients who died, and these are discussed in detail. A close correlation is found between autopsy findings and P.E.G.'s.



## **Congenital Heart Disease in Children under Two Years of Age**

### ***A Diagnostic Survey with Special Reference to Cardiac Catheterisation and Cine-Angiocardiography Used in a Series of Consecutive Cases***

by H. GØTSCHÉ, O. MORTENSEN and E. MOSEKILDE

*(Supplement 128)*

During the years 1956-1959 inclusive 201 children under 2 years of age were referred to hospital for investigation for congenital heart disease. In 31 cases, clinical and radiographic examination revealed normal conditions; in 13 cases, continued clinical observation was indicated. Congenital heart disease was revealed in 157. Of these, 59 were not subjected to cardiac catheterisation, either because they were in a very poor condition and died before the examination could be accom-

plished, or because their condition was so good that catheterisation, with advantage, could be postponed until the age of 3-4 years. Catheterisation was performed in 98, including 44 who were also subjected to angiocardiography. Of these 98 cases, 80 were fully clarified; the principal anomaly was disclosed in nine; the diagnosis was uncertain in seven (including three in whom catheterisation was unsuccessful); erroneous diagnoses were made in two. Death occurred in one case.

## **Preluxation of the Hip Joint. Diagnosis and Treatment in the Newborn and the Diagnosis of Congenital Dislocation of the Hip Joint in Sweden during the Years 1948-1960**

by KURT PALMÉN

*(Supplement 129)*

Since 1950 the author has examined the hip joints of all newborn, i.e. 12,394 children, at the obstetric clinic of the Central Hospital in Falköping, using the abduction manoeuvre described by Ortolani, which from 1956 onwards has been complemented with a subluxation provocation. Up to and including 1960, 70 cases

with positive symptoms were diagnosed in this way. These were treated immediately with fixation in an abduction position on a wooden board. The method of examination and the treatment are described.

At a re-examination in 1961, out of 39 cases who were at least 3 years old, 34 (87.2 %) showed clinically and roentgeno-



logically normal joints. In the remaining 5 cases the continued development of the joint will probably be normal in a further 2. The percentage of normal hips will then be 92.3 %. One case had dysplastic changes to a somewhat greater degree, and two showed results of capital necrosis. Their future development is at present uncertain.

The cases thus diagnosed are regarded as representing a stage of preluxation in the newborn. Left untreated, a number of these cases may heal spontaneously, and others may give rise to subluxation or luxation during the first to second year of life, in milder cases not until later in childhood, or adult years in the form of arthrosis deformans (osteoarthritis).

A critical study is made of the roentgen examination of the newborn, and in particular its sources of error. This examination revealed nothing in cases of preluxation in addition to that which could be established clinically. Roentgen examination of these cases before the commencement of treatment is therefore considered unnecessary.

An examination of a normal material of newborn was made in respect to the possible degree of abduction in the hip joints and the symmetry of the skin fold on the medial side of the thigh. All had an abduction of at least 70°. Only 39.6 % had symmetrical thigh folds. 27.6 % had no folds at all, these being mainly children with a low birth weight. Asymmetry of the skin folds thus does not appear to be a symptom of value in the diagnosis.

Most cases of preluxation have normal abduction, and many have increased flaccidity of the joint.

At the suggestion of the author the examination of the hip joints was in-

troduced in January 1953 into the routine examination of all newborn children at the majority of obstetric clinics having pediatric consultants. Up to and including 1960 approximately 415,500 newborn children were examined, constituting 49.5 % of all children born alive in Sweden during this period. During 1959 and 1960 approximately 67 % were examined. This material was analyzed. Altogether 894 cases of preluxation were diagnosed (=2.2%). During the later years this frequency was about 3%. In those clinics which also used the subluxation provocation method in the examination during the later years, the frequency was about 5-6 %. No obvious geographical differences in the frequency within Sweden was found in this material.

In order to study how the diagnoses made at the obstetric clinics have affected the frequency of luxation during later childhood, and thereby the usefulness of this diagnosis, all cases of luxation of the hip joint in children treated at the orthopedic clinics in Sweden during the years 1948-1960, 1486 cases altogether, were recorded. (In Sweden practically all cases of luxation are treated at orthopedic clinics.) The ages of the children at the time the diagnosis was made, and the reasons leading to the examination of the hip joints, are given.

During the later years an increasingly large number of newborn children were transferred to the orthopedic clinics, but in spite of this there was no marked increase in the number of cases treated annually at these clinics.

Approximately 62 % of the preluxation cases were treated at the pediatric clinics, the majority as out-patients.

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Of the cases of luxation admitted to orthopedic clinics during the years 1948-1950, none had been diagnosed when newborn, and 85 % were not diagnosed until after the age of 1 year. Ninety-seven per cent underwent the customary prolonged treatment with plaster.

Of the 229 cases treated during the years 1959-1960, 69 % were diagnosed at obstetric clinics, and only 23 % after the age of 1 year. It was possible for 58 % to be treated as out-patients, with a simple method of fixation in an abduction position for a period, in the majority of cases, of only a few months. Thirty-nine per cent were treated with plaster.

The material from the orthopedic clinics was compared with that from the obstetric clinics, and in the cases of luxation from the orthopedic clinics, inquiry was made as to the obstetric clinic at which the child was born. It was thereby found that during the years 1953-1960, 114 children were admitted, who were not diagnosed as preluxations when newborn, but who were born at those clinics where

the hip joints were examined as routine. Ten cases had been transferred to a pediatric clinic because of prematurity, asphyxia or malformations, and had possibly therefore had no hip joint examination immediately. It was not possible to verify with certainty whether all of the other cases had been examined when newborn.

An obvious tendency was observed in the number of cases of later manifested luxation which was not discovered in the neo-natal period, to decrease with the years, when the interest and experience of the examiners had extended.

The material from those clinics which have shown especial interest and have considerable experience, reveals that at least 90 % of those dislocations which may be expected to occur during later childhood years, can be diagnosed as preluxations during the first week of life. In this way they are also available for immediate treatment with simple fixation in the abduction position, and in probably at least 90 % the subsequent development of the joint is completely normal.

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*Subscription Price 50 Swedish kronor. Single copies 15 Swedish kronor.*

Subscription should be addressed to ALMQVIST & WIKSELL, International Booksellers,  
26 Gamla Brogatan, Stockholm, Sweden.

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1. BROCK, J.: *Biologische Daten für den Kinderarzt*, vol. 2, p. 330. Springer-Verlag, Berlin, 1954.
2. STEVENSON, S. S.: Possible ariboflavinosis in a premature infant. *Yale J Biol Med*, 14: 403, 1943.

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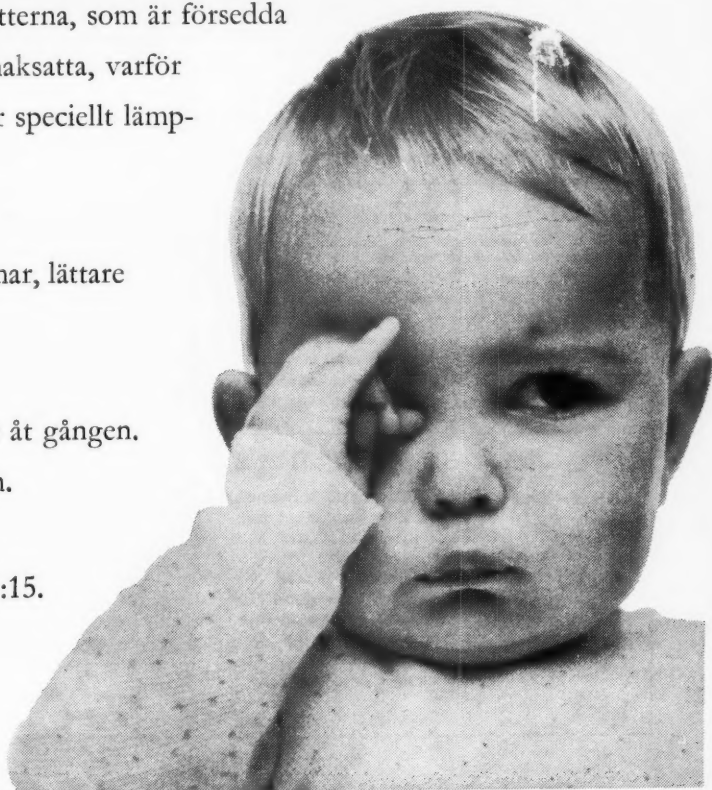
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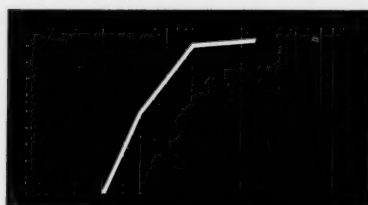
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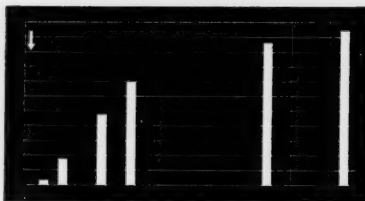
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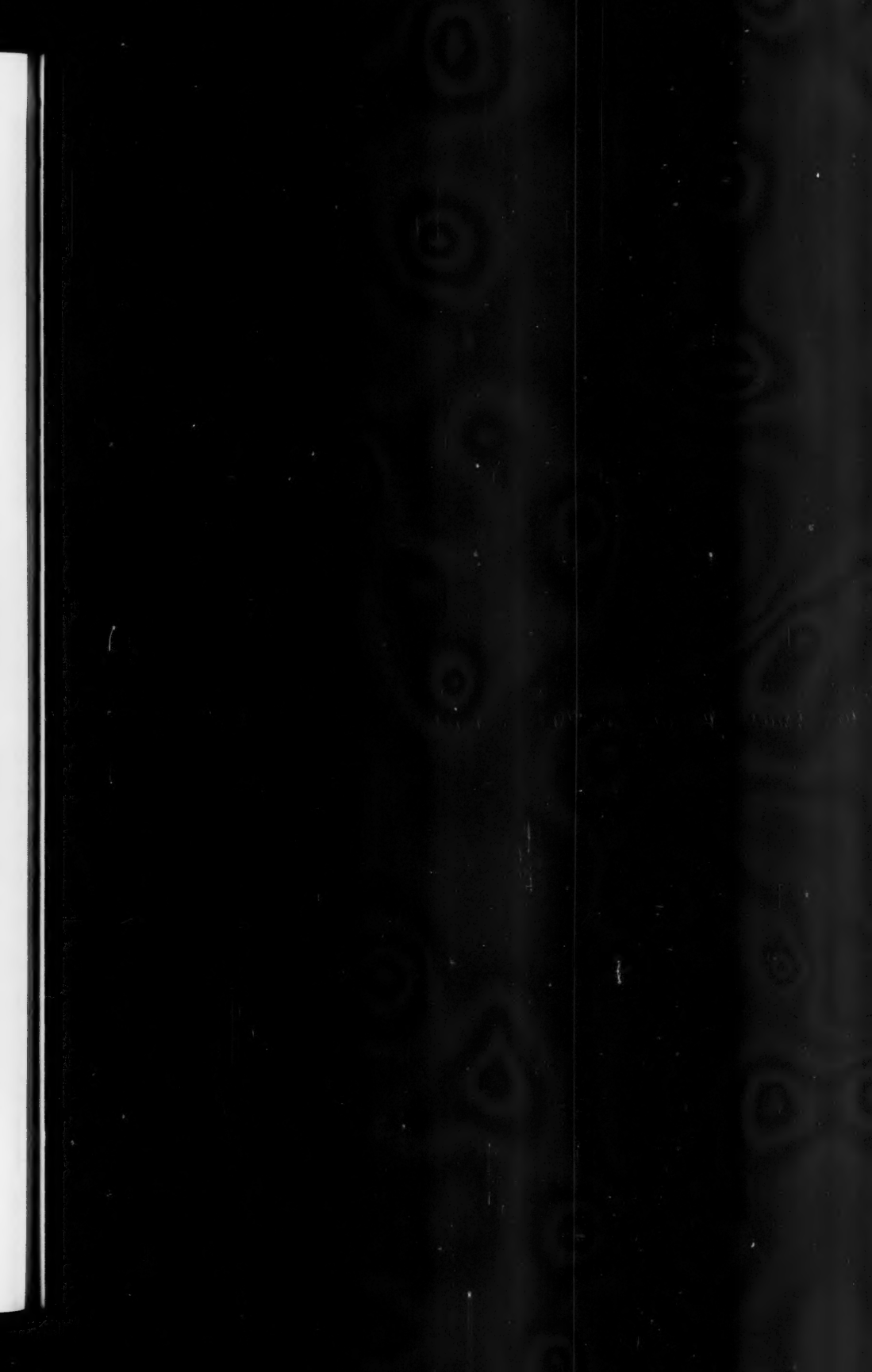


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